Effect of Pretreatment on the Breakdown of Lignocellulosic Matrix in Barley Straw as Feedstock for Biofuel Production

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Abstract

Lignocellulosic biomass is composed of cellulose, hemicellulose, lignin and extraneous compounds (waxes, fats, gums, starches, alkaloids, resins, tannins, essential oils, silica, carbonates, oxalates, etc). The sugars within the complex carbohydrates (cellulose and hemicellulose) can be accessed for cellulosic bioethanol production through ethanologenic microorganisms. However, the composite nature of lignocellulosic biomass, particularly the lignin portion, presents resistance and recalcitrance to biological and chemical degradation during enzymatic hydrolysis/saccharification and the subsequent fermentation process. This leads to a very low conversion rate, which makes the process uneconomically feasible. Thus, biomass structure requires initial breakdown of the lignocellulosic matrix.

In this study, two types of biomass pretreatment were applied on barley straw grind: radio-frequency (RF)-based dielectric heating technique using alkaline (NaOH) solution as a catalyst and steam explosion pretreatment at low severity factor. The pretreatment was applied on barley straw which was ground in hammer mill with a screen size of 1.6 mm, so as to enhance its accessibility and digestibility by enzymatic reaction during hydrolysis. Three levels of temperature (70, 80, and 90^{o}C), five levels of ratio of biomass to 1% NaOH solution (1:4, 1:5, 1:6, 1:7, & 1:8), 1 h soaking time, and 20 min residence time were used for the radio frequency pretreatment. The following process and material variables were used for the steam explosion pretreatment: temperature (140-180^{o}C), retention time (5-10 min), and 8-50% moisture content (w.b). The effect of both pretreatments was assessed through chemical composition analysis and densification of the pretreated and non-pretreated biomass samples. Results of this investigation show that lignocellulosic biomass absorbed more NaOH than water, because of the hydrophobic nature of lignin, which acts as an external crosslink binder on the biomass matrix and shields the hydrophilic structural carbohydrates (cellulose and hemicellulose). It was observed in the RF pretreatment that the use of NaOH solution and the ratio of biomass to NaOH solution played a major role, while temperature played a lesser role in the breakdown of the lignified matrix, as well as in the production of pellets with good physical quality. The heat provided by the RF is required to assist the alkaline solution in the deconstruction and disaggregation of lignocellulosic

biomass matrix. The disruption and deconstruction of the lignified matrix is also associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF and the ions and molecules from the NaOH solution and the biomass. The preserved cellulose from the raw sample (non-treated) was higher than that from the RF alkaline pretreated samples because of the initial degradation of the sugars during the pretreatment process. The same observation applies to hemicellulose. This implies that there is a trade-off between the breakdown of the biomass matrix/creating pores in the lignin and enhancing the accessibility and digestibility of the cellulose and hemicellulose. The use of dilute NaOH solution in biomass pretreatment showed that the higher the NaOH concentration, the lower was the acid insoluble lignin and the higher was the solubilized lignin moieties. The ratio of 1:6 at the four temperatures studied was determined to be the optimal. Based on the obtained data, it is predicted that this pretreatment will decrease the required amount and cost of enzymes by up to 64% compared to using non-treated biomass. However, the use of NaOH led to an increase in the ash content of biomass. The ash content increased with the decreasing ratio of biomass to NaOH solution. This problem of increased ash content can be addressed by washing the pretreated samples. RF assisted-alkaline pretreatment technique represents an easy to set-up and potentially affordable route for the bio-fuel industry, but this requires further energy analysis and economic validation, so as to investigate the significant high energy consumption during the RF-assisted alkaline pretreatment heating process.

Data showed that in the steam explosion (SE) pretreatment, considerable thermal degradation of the energy potentials (cellulose and hemicellulose) with increasing acid soluble and insoluble lignin content occurred. The high degradation of the hemicellulose can be accounted for by its amorphous nature which is easily disrupted by external influences unlike the well-arranged crystalline cellulose. It is predicted that this pretreatment will decrease the required amount and cost of enzymes by up to 33% compared to using non-treated biomass.The carbon content of the solid SE product increased at higher temperature and longer residence time, while the hydrogen and oxygen content decreased.

The RF alkaline and SE treatment combinations that resulted to optimum yield of cellulose and hemicellulose were selected and then enzymatically digested with a combined mixture of cellulase and β-glucosidase enzymes at 50^{o}C for 96 h on a shaking incubator at 250 rev/min. The glucose in the hydrolyzed samples was subsequently quantified. The results obtained confirmed the effectiveness of the pretreatment processes. The average available percentage glucose yield that was released during the enzymatic hydrolysis for bioethanol production ranged from 78-96% for RF-alkaline pretreated and 30-50% for the SE pretreated barley straw depending on the treatment combination. While the non-treated sample has available average percentage glucose yield of just below 12%.

The effects of both pretreatment methods (RF and SE) were further evaluated by pelletizing the pretreated and non-pretreated barley straw samples in a single pelleting unit. The physical characteristics (pellet density, tensile strength, durability rating, and dimensional stability) of the pellets were determined. The lower was the biomass:NaOH solution ratio, the better was the quality of the produced pellets. Washing of the RF-alkaline pretreated samples resulted in pellets with low quality. A biomass:NaOH solution ratio of 1:8 at the three levels of temperature (70, 80, and 90^{o}C) studied are the RF optimum pretreatment conditions. The higher heating value (HHV) and the physical characteristics of the produced pellets increased with increasing temperature and residence time. The steam exploded samples pretreated at higher temperatures (180^{o}C) and retention time of 10 min resulted into pellets with good physical qualities.

Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) was further applied on the RF alkaline and SE samples in light of the need for rapid and easy quantification of biomass chemical components (cellulose, hemicellulose, and lignin). The results obtained show that the FTIR-PAS spectra can be rapidly used for the analysis and identification of the chemical composition of biofuel feedstock. Predictive models were developed for each of the biomass components in estimating their respective percentage chemical compositions.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

1. Introduction and Objectives

1.1 Introduction

Biomass resources comprise of forest and agricultural residues. It also includes industrial and municipal solid wastes. Biomass as a renewable source of energy is environmental benign with neutral carbon footprint (Liu 2005; Lynd 1999; Cadenas and Cabezudo 1998). Biomass is readily available worldwide in large quantities and it is considered to be a good resource to complement fossil fuels, hence, enhancing the sustainability of energy supply (Carolan et al. 2007; Lynd 2005; Lynd 1999). The combustion of petroleum-based fuels poses a threat to environmental sustainability. The use of these types of energy is responsible for the atmospheric pollution resulting to the emission of greenhouse gases (GHG) (Demirbas et al. 2009). The scientific community attributed the degradation of the atmosphere (climate change and global warming) to the combustion of these fuels (Fiona et al. 2007). The increasingly world population and industrialization has also demanded alternative sources of energy.

Bioethanol is an alcohol produced via fermentation of simple sugar components of biomass and starch crops. Presently, it is made mostly from sugar and starch crops. These categories of crops that can be used both as food and as biofuel are usually referred to as ‘first generation’ bioenergy crops (Taylor 2008). Bioethanol is made from corn in the United State, sugar cane in Brazil, wheat grain in the UK, and wheat and barley grains in Canada. These are staples foods in some countries and could cause shortage of foods because of the competition between food and energy. In recent time, the target is to produce renewable energy from lignocellulosic biomass which comprises agricultural and forest residues, and non-food dedicated energy crops (these are biomass that is mostly plant cell walls with high carbon content). Such dedicated bioenergy

crops (Miscanthus and short rotation woody crops such as willows, poplar) that has no food use are usually referred to as 'second generation' bioenergy crops (Taylor 2008).

Agricultural crop residues such as straws are considered as good feedstocks for biofuel applications due to the low nutritional value when used as feed for animals (Tavakoli et al. 2009). Saskatchewan Ministry of Agriculture reported that in 2013, Canada produced about 69.6 million tonnes (mt) of wheat, canola, barley, and oat, with Saskatchewan production at 33 mt. Barley production was at 3.4 mt and 10.2 mt for Saskatchewan and Canada, respectively. Montane et al. (1998) reported that an average ratio of 1.3 kg of straw per kg of wheat grain harvested is obtained for most common varieties of wheat. This translates to total production in 2013 to about 42.8 mt and 90.5 mt of straws for Saskatchewan and Canada, respectively.

Lignocellulosic biomass feedstock contains three main components: cellulose, hemicellulose, and lignin. The association among these biomass components makes the production of bioethanol from lignocellulosic biomass tedious and uneconomically feasible (Kumar et al. 2009). This necessitates the initial pretreatment to enhance the release of the energy potentials present in lignocellulosic biomass. Cellulose and hemicellulose can be hydrolyzed into simple sugars, mainly glucose and xylose, respectively, lignin cannot be fermented, but it can be used to provide process heat during the fermentation process (Cadenas and Cabezudo 1998). Some of the pretreatment methods include: ammonia fibre explosion (AFEX), steam explosion, microwave-chemical, biological, acid hydrolysis, wet oxidation, and supercritical CO_2 explosion (SC- CO_2) pretreatment (Montane et al. 1998; Okano et al. 2005; Palonen et al. 2004; Saha et al. 2005; Zhu et al. 2006; Kim and Hong 2001; Alizadeh et al. 2005).

Biomass in its natural form is loose and bulky, and cannot be handled in a cost competitive manner. A densified biomass occupies less volume and decreases the transportation cost relative to biomass in its original state (Sokhansanj et al. 2005). Dense biomass can be handled as grains and stored safely and at low cost using well known established handling systems for grains (Sokhansanj et al. 2005). Sokhansanj et al. (2005) reported that the bulk density of a dried straw is as low as 40 kg/m^3 as compared to the bulk density of pelletized grasses which can be as high

as 1250 kg/m3 (Adapa et al. 2002). Biomass including straws and stover are difficult to densify and requires the application of expensive binders such as Collagen protein, hydrated lime, lignosulfonate, bentonite and pea starch (Sokhansanj et al. 2005; Tabil 1996). Research has shown that modifying the structure of cellulose-hemicellulose-lignin matrix through pretreatment techniques can improve the natural binding characteristics of the lignocellulosic biomass straw (Sokhansanj et al. 2005).

1.2 Research Objectives

The main objective of this research is to investigate the pretreatment of lignocellulosic biomass (barley straw) to improve the accessibility of the energy potentials (cellulose and hemicellulose) and enhance densification process.

The following specific objectives have been defined for this present research work:

1. to explore the potentials of radio frequency (RF) heating as a pretreatment method for the deconstruction and disaggregation of lignocellulosic biomass;

2. to predict the degree of accessibility and digestibility of cellulose and hemicellulose for the subsequent stage of enzymatic hydrolysis/saccharification;

3. to assess the operating conditions for steam explosion (SE) treatment of biomass straw at low severity factor and conditions with minimal production of inhibitors (furfural);

4. to validate and evaluate the readily available enzyme digestible glucose (cellulose) in the RF alkaline and SE pretreated and non-treated samples

5. to study and rapidly estimate the relative chemical composition of lignin, cellulosic and hemicellulosic polymers in pretreated and non-treated lignocellulosic barley straw using Fourier Transform Infrared-Photoacoustic Spectroscopy, and also to develop an easy and non-laborious approach for the quantitative measurement of lignocellulosic biomass chemical compositions

6. to investigate the effects of RF alkaline and SE pretreatments on the physical characteristics (density, tensile strength, durability, and dimensional stability) of pelletized barley straw, which could be used as feedstock for the production of bio-energy via thermochemical process.

1.3 Organization of the Thesis

The thesis is organized according to the University of Saskatchewan guidelines for manuscript based theses. This implies that the experimental data and analysis presented in this thesis have been published or in-press with peer-reviewed journals. These manuscripts were written and submitted for publication as each stage of the Ph.D. project was completed. Chapter 2 of this thesis has been published as a book chapter with Nova Science Publishers, Inc. The manuscripts presented in Chapters 3, 4, 6 and 7 have been published (or in-press) in peer-reviewed journals. The manuscript in appendix A to B and Chapter 5 has been presented and accepted as a conference proceeding paper. In each manuscript based-chapter, the contribution of the Ph.D. candidate and the contribution of the paper to the overall study are discussed in addition to the manuscript itself. Chapters 1, 8 and 9 are original text in this thesis included to introduce the subject matter and discuss the outcome of the project. Chapter 10 contains all the references for chapters 1 to 7, with any useful supplementary material given in the Appendices.

1.4 Manuscript Content of the Thesis

The present Ph.D. research program has resulted to bridge of knowledge and development of new procedures for the pretreatment of lignocellulosic biomass leading to the cellulosic ethanol production. In appendix A, the dielectric properties of lignocellulosic biomass barley straw and sodium hydroxide relevant to radio frequency pretreatment processes were measured using the precision LCR meter and liquid test fixture. The effects of the process temperature, biomass moisture content, sodium hydroxide concentration, biomass screen size, soaking time, and ratio of biomass to alkaline solution on dielectric constant, dielectric loss factor, and power penetration depth were investigated. The results obtained was used to determine and establish relationships among the above mentioned variables and subsequently applied in the design,

formulation, and optimization of the functional RF pretreatment process used in chapter 3. The effect of the RF alkaline pretreatment was evaluated through chemical composition analysis of the pretreated and non-treated biomass samples using the National Renewable Energy Laboratory Standard (NREL). The physical characteristics of biomass barley straw grind: particle, bulk, and solid densities, inter-particle porosity, porosity within particles, and the energy consumption required for the RF pretreatment were also measured in chapter 3. The biofuel industry can used this data in the design of a pilot-scale RF alkaline biomass pretreatment plant for the production of bioethanol. They can also use it to predict the cost of the enzymes required in the subsequent stage of enzymatic hydrolysis. In chapter 4, the pretreatment of biomass barley straw grind using SE at low severity factor was successfully performed as a comparison with the RF alkaline technique. In chapter 5, the RF alkaline and SE treatment combinations that resulted to optimum yield of cellulose and hemicellulose were selected and then enzymatically digested with a combined mixture of cellulase and β-glucosidase enzymes. The glucose in the hydrolyzed samples was subsequently quantified. The procedure developed in this study can easily be adopted and applied by the bioethanol industry to quickly estimate the accessibility and digestibility of the simple sugars during the subsequent stage of enzymatic hydrolysis. Chapter 6 presents structural and chemical characteristics of agricultural biomass. A procedure to rapidly quantify the lignocellulosic chemical composition of RF, SE, and non-treated barley straw using Fourier Transform Infrared Spectroscopy (FTIR) was developed. This can easily be extended to any form of lignocellulosic biomass. Finally in chapter 7, a further study was performed to evaluate the effects of RF alkaline and SE pretreatments. Practical problems associated with particle disintegration and fractures relating to density, tensile strength, durability, and dimensional stability of densified barley straw were investigated. This procedure could be used by pellet manufacturers to optimize the production of bioenergy via thermochemical process. In appendix B, further studies was performed to gain understanding on how operating and material variables could affect the thermal characteristics of RF heating, thereafter, the thermal conductivity, specific heat, and thermal diffusivity of lignocellulosic biomass barley straw grind as a function of temperature, ratio of biomass:NaOH solution (liquid content), and hammer mill screen size (bulk density) were determined.

Chapter 2

2. Lignocellulosic Biomass: Feedstock Characteristics, Pretreatment Methods and Pre-Processing for Biofuel and Bioproduct Applications, U.S. and Canadian Perspective

A similar version of this chapter has been published as a book chapter with Nova Science Publishers, Inc.:

- Iroba, K.L. and L.G. Tabil. 2013. Lignocellulosic biomass: feedstock characteristics, pretreatment methods and pre-processing for biofuel and bioproduct applications, U.S. and Canadian perspective. In Biomass Processing, Conversion and Biorefinery, ed. B. Zhang, Y. Wang, 61-98. New York, NY: Nova Science Publishers, Inc.

Contributions of this Paper to Overall Study

This exhaustive literature review was collected and developed to serve as a reference resource when looking for information on the potentials of lignocellulosic biomass, its advantages as a source of renewable energy over the conventional fossil fuels, different pretreatment methods of biomass, the merits and demerits of the various pretreatment methods, and the characteristics of lignocellulosic biomass as a densified product based on the material and process variables. The whole manuscript was written by Kingsley Iroba, while Dr. Lope Tabil provided editorial input.

2.1 Abstract

Renewable energy using lignocellulosic biomass is believed to become one of the major energy resources in the near future to deal successfully with global warming and depletion of conventional fossil fuel resources. This will to a large extent save our environment and human health. The challenges associated in the production of bioethanol from lignocellulosic biomass must be critically addressed so as to enhance the digestibility and accessibility of cellulose and hemicellulose, and the subsequent conversion to simple sugars usable by fermentation yeasts. Effective conversion relies on a number of factors: the biomass feedstock composition and structure, particle size, pretreatment method used, types and loading of enzymes. The highly structured crystalline cellulose acts as an obstacle to hydrolysis. Cellulose and hemicellulose are to a great degree protected from enzymatic attack. This inaccessibility to attack is primarily a result of the association of these polysaccharides with lignin, which acts as a barrier, shielding the polysaccharides. Optimization of the pretreatment, hydrolysis, and fermentation processes with minimal production of inhibitors will contribute immensely to an efficient and cost-effective biorefinery industrial process. To mitigate the difficulty created by the bulky, loose, and disperse nature of lignocellulosic biomass, there is a need for pre-processing and densification for easy, economical and efficient transportation, handling, and storage. It increases mass per unit volume and improves the convenience and accessibility of lignocellulosic biomass feedstock due to the uniform shape and size.

2.2 Introduction

The world's major energy reliance is on fossil fuels. These energy sources are not renewable, because they take several years to be formed. For decades, the use of fossil fuels has been a major factor of environmental degradation and pollution. The heavy dependence on petroleum is extremely a serious issue on world's energy security; there might be increase in energy demand with the geometric increasing world population, and there is also the fear of depletion in the supply of petroleum (Mabee et al. 2005). The dominant energy structure reliance on coal in most parts of the world causes serious environmental problems. To reduce the dependence on fossil

fuels, there is tremendous interest and emphasis towards sustainable and environmentally friendly sources of alternative fuels (Fiona et al. 2007).

The present chapter aims to examine the economic and environmental benefits of biofuels and review the work that has been done in the past on biomass feedstocks. This chapter also sheds light on some of the practical approaches and methods that have been studied and adopted in the pretreatment, pre-processing, and densification of lignocellulosic biomass prior to the production of lignocellulosic biofuel. The main bottlenecks involved in lignocellulosic biomass processing, which do not encourage cost-effective and competitive production of biofuel are also discussed.

2.2.1 Fossil Fuel and the Environment

It is generally accepted by the scientific community that global climate change is due to the forced warming from greenhouse gases (GHGs) resulting to the depletion of the ozone layer in the stratosphere, which acts as shield to the ultraviolet radiation from the sun (Fiona et al. 2007). The United Nation's Intergovernmental Panel on Climate Change (IPCC-UN 2007) reported that the forced warming of the climate system is as a result of the observed increase in anthropogenic GHG concentrations. In the last century (twentieth century), the average global temperature increased by 0.74^{o}C and sea level rise amounted to 17 cm. This is due to the thermal expansion of the ocean and melting of ice across the world (IPCC-UN 2007). According to long term forecasts, if this increase in temperature continues, the world will experience more floods, storms, heat waves, and droughts. This exposes low lying coastal countries with land surface about a meter or two above sea level to serious danger with life-threatening consequences (IPCC-UN 2007). Major GHGs include carbon dioxide, methane and nitrous oxide. The present atmospheric CO_2 increase is primarily connected to anthropogenic emissions. The burning of fossil fuels accounts for about three-quarters of the overall GHG emissions (Environment Canada 1996). Between 1980 and 1989, human activities caused annual released of an estimated 7.1 billion tonnes of carbon into the atmosphere as carbon dioxide (Environment Canada 1996). Majority of this, 5.5 billion tonnes per year on average resulted from fossil fuels combustion, with little amount from cement production. The remaining 1.6 billion tonnes was as a result of the contribution from burning and clearing of tropical forests (Environment Canada 1996).

Increase in the combustion of fossil fuels to meet global energy needs will substantially increase the GHG emissions. In addition to these negative effects, fossil fuels combustion is a major contributor to acid rain formation (Environment Canada 1996). The large amount of sulfur and nitrogen contents in fossil fuels produce sulfur dioxide and nitrous oxides when combusted. This results to the formation of acid rain which can damage fresh water sources, forests, soils, and buildings and negatively affect human health (Demirbas 2004).

2.2.2 Biomass as a Potential Source of Energy

To overcome the aforementioned problems, there is a need for a more reliable, sustainable, renewable, and environmentally friendly energy source. Lignocellulosic biomass (LB) feedstock is one potential source for alternative energy. As a result of the extremely large quantity, availability, and renewable characteristic of LB, the efficient utilization as energy source has been receiving great attention. LB has annual production of approximately 200 billion tonnes worldwide (Zhang 2008), and is considered as the best option with the highest possibility to add to the energy needs of the present society and ensure fuel supply in the future for both developing and industrialized nations (Demirbas et al. 2009). In other to avoid the competition between food and energy, current attention has been focused on the agricultural residues (non-edible portion) as source of bioenergy and bioproducts, since they are readily available worldwide at low cost (Lynd 1999; Lynd 2005; Carolan et al. 2007). Therefore, it is of paramount importance to fully harness the potentials of LB. Other alternative renewable energy sources are geothermal, hydroelectric, solar, and wind. These are vital components of a long-term environmentally sustainable solution to complement the use of fossil fuels. Using these alternative sources of energy will enhance diversity, energy security, sustainability, and improve air quality, as well as mitigate the harmful effects of greenhouse gases (Hoekman 2009).

Lignocellulosic biomass refers to the organic matter which comprises agricultural crop residues (such as straw/stalk, stover and bagasse), forest residue (woody biomass such as sawdust), agricultural dedicated energy crops (short-rotation woody crops such as poplar, willow, switchgrass), municipal solid waste, animal wastes, waste from food processing, and aquatic plants and algae (Lynd 2005; Demirbas 2001; Tabil 2009). These are the most important biomass

energy sources which can be used for energy and in a number of different applications (Demirbas 2001; Tabil 2009).

The carbon dioxide released during the combustion process or usage of biofuel is compensated by the carbon dioxide intake by plants from the atmosphere during the process of photosynthesis over their growth cycle, which converts light energy to chemical energy and stores it as carbohydrates as shown below:

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2 \quad (2.1)$$

Hence, bioenergy have the potential for carbon sequestration to reduce the negative effects of global warming. Thus, they are classed as a carbon-neutral fuel. Above all, there is no fear of depletion; rather it will encourage farmers to produce more food with the intention of making more profit from the residues, and at the same time creating more job opportunities and providing regional economic development (Demirbas 2001; Sneller et al. 2006).

2.2.3 Ethanol Benefits and Considerations

Besides the benefits mentioned previously, bioethanol have some other inherent advantages over gasoline. Table 2.1 compares the fuel properties of bioethanol and gasoline. Flash point (the lowest temperature at which a material can vaporize to produce an ignitable mixture in air) of bioethanol (12.78^{o}C) is much higher than that of gasoline (-42.78^{o}C) (U.S. DOE). This is an indication of the safety level of bioethanol usage. Gasoline is a dangerous liquid in an accident or in a crash, because it can explode or ignite. Producing, refining, transporting, and using gasoline can lead to severe environmental discharge of pollutants (U.S. DOE/Energy).

Table 2.1 Fuel property comparison for ethanol and gasoline (U.S. DOE)

Property	Ethanol	Gasoline
Chemical Formula	C_2H_5OH	C4 to C12
Molecular Weight	46.07	100–105
Carbon	52.20	85–88
Hydrogen	13.10	12–15
Oxygen	34.70	0
Specific gravity, 15.56℃/15.56℃	0.80	0.72–0.78
Density, kg/L @ 15.56℃	0.79	0.72-0.78
Boiling temperature, ℃	77.78	26.67–225
Reid vapor pressure, kPa	15.86	55.16–103.42
Research octane no.	108	90–100
Motor octane no.	92	81–90
(R + M)/2	100	86–94
Cetane no.(1)	--	5–20
Fuel in water, volume %	100	Negligible
Water in fuel, volume %	100	Negligible
Freezing point, ℃	-114	-40
Dynamic viscosity, 15.55^{o}C	$1.18x10^{-3}$	$(3.7–4.4)\ x10^{-3}$*
Flash point, closed cup, ℃	12.78	-42.78
Auto-ignition temperature, ℃	422.78	257.22
Specific heat, kJ/kg K (C_p)	1.88	2.22

*Calculated

The auto-ignition temperature (which does not require an ignition source) of bioethanol (422.78^{o}C) is also much higher than that of gasoline (257.22^{o}C) (U.S. DOE/Energy). This also shows that bioethanol is reliably safer to consumers than gasoline. The characteristic temperature at which liquids turn into solids (known as freezing point) is much lower than that of the gasoline (U.S. DOE). This shows that bioethanol can be more effectively used in the cold/artic regions of the world without impeding engine performance. The research and motor octane number of bioethanol (108 and 92, respectively) is higher than gasoline (90-100 and 81-90, respectively) (U.S. DOE/Energy). The higher the octane number, the higher the amount of compression that the fuel can successfully withstand before detonating (U.S. DOE). In principle, fuels with a higher octane rating are used in high-compression engines which are generally accompany with higher performance. The use of gasoline with low octane numbers generally results to engine knocking (U.S. DOE). A liter of bioethanol contains less energy as compared to a liter of gasoline, which depends on the blend (U.S. DOE/Energy). E85 (85% bioethanol/15% gasoline

on a volume basis) has about 27% less energy per liter than gasoline. However, because bioethanol is a high-octane fuel, it has the advantage of increased vehicle power and performance (U.S. DOE/Energy). Sheehan et al. (2004) reported that for each kilometer fueled by bioethanol share of E85, the vehicle consumes 95% less fuel as compared to a kilometer driven in the same vehicle on gasoline. Greenhouse gas emissions (fossil CO_2, N_2O, and CH_4) on a life-cycle basis are reduced by 113% using E85 (Sheehan et al., 2004). Sheehan et al. (2004) reported that displacing gasoline with E100 decreases fossil CO_2 emissions by 267 g of CO_2/km

2.2.4 Biomass Resources

2.2.4.1 A Canadian Focus

Sokhansanj et al. (2006) studied the production and distribution of cereal straw (wheat, barley, oats, and flax) in the Canadian prairies over a period of 10 years (1994-2003). They reported that the provinces of Alberta, Saskatchewan, and Manitoba collectively produced approximately 37 million tonnes of wheat, barley, oat, and flax straw annually. After grain harvest, some amount of the available straw is always left on the field to maintain soil health and fertility and to avoid soil erosion (water and wind). Excess straw available can be used in a sustainable way for other economic purposes like livestock feeding and bedding, while the rest is bunched and the piles are burn on the farm land (Sokhansanj et al. 2006). The net average available straw from the prairies that was burnt annually after satisfying soil conservation and livestock requirements is estimated at 15 million tonnes, which can sometimes be as high as 27.6 million tonnes and as low as 2.3 million tonnes depending on the year's harvest (Sokhansanj et al. 2006). The results of this study show that a good portion of crop straw resources is lost, with serious environmental pollution. This implies that straws (agricultural biomass residues) that remain as waste from farms and commercial crop processing plants have minor or no inherent value, which normally presents a disposal problem (Tabil et al. 2011). Lignocellulosic agricultural biomass residues constitute surplus, cheap and readily available source of environment friendly and renewable energy (Lynd 1999; Liu 2005). Lafond et al. (2009) studied the effects of straw removal from the agricultural field through baling and measured the long-term effect on soil quality and wheat production on the Canadian prairies. It was reported that there was a negligible effect on the amount of soil organic carbon and soil organic nitrogen. These researchers concluded that there is great

potential to harvest cereal crop residues with a baler for ethanol production or other industrial purposes without having negative effects on the soil quality and productivity, only if acceptable and satisfactory soil and crop management practices are applied.

Canadian governments at the national level and some provinces (including Alberta, British Columbia, Maritimes, Ontario, Quebec, and Saskatchewan) have shown considerable support and efforts toward developing and promoting an industry-led approach to the establishment and development of the bioproducts industry (Mabee 2005). According to Saskatchewan Ministry of Agriculture (2008), Saskatchewan alone produced 12.44 million tonnes of wheat, 5.63 million tonnes of canola, 4.59 million tonnes of barley, and 2.3 million tonnes of oat in 2008. The Food and Agriculture Organization (FAO-UN 2007) reported that Canada produced about 21 million tonnes of wheat, 11 million tonnes of barley, and 5 million tonnes of oat in 2007. It should be noted that straw yield depends on the specific varieties and is mostly affected by agronomic, environmental, and climatic factors. Montane et al. (1998) cited that an average ratio of 1.3 kg of straw per kg of wheat grain harvested is obtained for most common varieties of wheat. The associated issues of biodiversity and soil conservation, however, will limit the removal of agricultural residues (Mabee 2005).

Canada plays a very significant role in the development of biorefinery sector because of its contribution as a predominant supplier or source of biomass feedstock. Canada has 402.1 million hectares of forest and other wooded land which represents 10% of the world's forest cover and 30% of the world's boreal forest (NRcan 2009). There is great potential for the forestry sector to supply part of or the entire feedstock requirement for bioenergy, biofuel and bioproduct sectors. Canadian forests predominantly supply more than 200 million m^3 of biomass annually via commercial operations (NRcan 2009; FAO-UN 2009). This makes Canada the second largest producer of woody lignocellulosic biomass in the world, behind the United States (NRcan 2009; FAO-UN 2009).

The comparatively high availability of Canadian biomass is an indication that Canadian agriculture and forestry sectors has great potential of leading the world in the development of

biorefinery sector, while meeting food and feed demands (Mabee 2005). This can generate an additional economic activity by potentially creating both direct and indirect job opportunities while making agriculture and forestry more stable and sustainable industries, and enhance tax revenue generation for provincial and federal governments. Hence, it is of paramount importance to optimize the extraction process of the necessary sugars for the production of bioethanol.

2.2.4.2 Biomass Resources in the U.S.

The oil crisis of the 1970s triggered an interest in the United States (U.S.) in building domestic and environmental friendly energy resources that could decrease the country's reliance on non-renewable and foreign energy supplies (U.S. DOE/Energy). Recently, in the U.S., the use of biofuels for transportation has become subject of extreme policy debate and action resulting from the combination of the following factors: a) unstable political environment in oil producing countries that do not have good relationship with the U.S.; b) fluctuating energy prices; c) increasing global demand for fossil fuels resulting from the geometric increasing population; and d) issues of global warming resulting from the use of fossil fuels (U.S. DOE/Energy; Sheehan et al. 2004; MacLean and Lave 2003). More than 50% of the oil consumed in the U.S. is from foreign countries and about two-thirds of U.S. petroleum demand is use in the transportation sector (U.S. DOE; U.S. DOE/Energy; Sheehan et al. 2004).

In the U.S., bioethanol is produced primarily from starch in corn kernels. Sheehan et al. (2004) reported that in 2002, 32 million hectares of corn grain were planted in the U.S. This amounts to a potential savings of approximately 740,000 barrels of crude oil/day, a savings of about 4% of the current U.S. demand for oil (Sheehan et al. 2004). However, it offers the possibility to enhance oil savings which can be compared with the 800,000 barrels/day in foreign oil savings forecasted for the operation of the Alaskan Natural Wildlife Reserve for oil drilling and exploration (Sheehan et al. 2004; U.S. DOE 2002).

Fortenbery and Park (2008) reported that in 2005, majority of the 15.14 billion liters of bioethanol produced came from 13% of the U.S. corn crop (36 billion tonnes of corn grain). In 2006 and 2007, the bioethanol produced in the U.S. exceeded 18.92 billion and 41.64 billion

liters, respectively, estimated to have used over 20% of domestic corn production (Fortenbery and Park 2008). America uses close to 511-530 billion liters of gasoline a year (Renewable fuels association 2012). The Renewable Fuels Association's (2012) calculated that in 2011, the bioethanol replaced gasoline from imported oil by more than 48.15 billion liters, which represents about 25% of locally processed and refined motor fuel for gasoline engines. With the increase in corn use by the bioethanol industry, a serious concern has developed with respect to bioethanol's effect on corn price. Corn is a second staple food in some countries; therefore, the use of corn grains for energy creates competition between food and energy. Looking at the numbers and impacts that corn makes, it is clear that corn is not the only solution to U.S. energy security issues. The U.S. needs to explore more of the agricultural residues (lignocellulosic biomass) to avoid such competition in future. In 2010, the U.S. produced about 316 Mt (million tonnes) of corn, 60 Mt of wheat, 4 Mt of barley, and 1.2 Mt of oats (FAOSTA). Bioethanol from lignocellulosic biomass has been considerably accepted to be more promising from a sustainability perspective, due to the noticeably lower life cycle GHG emissions compared to grain bioethanol (U.S. DOE/Energy; MacLean and Lave 2003; Wu et al. 2006). Perlack et al. (2005) estimated that up to 823 Mt of cellulosic biomass could be attainably collected from agricultural land within the next 35-40 years in the U.S. The various existing sources of cellulosic biomass (lumber industry waste and woody biomass, forage crops, animal manure, industrial waste, municipal solid waste (MSW) [trash or garbage which emanates from homes, schools, hospitals, and businesses], and crop residues are the potential candidates for the bioenergy industry (Perlack et al., 2005). The most abundant sources of biomass currently available for the production of bioethanol in the U.S. are crop residues. These could contribute about 446 million dry tonnes of biomass, which will amount to about 54% of the total cellulosic biomass sources in the U.S. (Sheehan et al., 2004; Perlack et al., 2005). In 2010, about 250 Mt of MSW was generated in the U.S. (U.S.-EPA 2012). Graham (2003) reported that the U.S. produced 521 million dry tonnes of agricultural waste (urban wood waste, mill waste, crop and forest residues, black liquor/pulp by-product, manure and bio-solids). The statistics above shows that the U.S. has a good potential for bioethanol production.

2.3 Structure and Compositions of Lignocellulosic Biomass

2.3.1. Challenges Associated with Lignocellulosic Biomass

Despite all the enormous benefits of using LB as bioenergy source, there are also some challenges that need to be overcome so as to make the process economically feasible and lucrative for investors, as well as to make biofuel easily affordable by the consumers. Lignocellulosic biomass is a complex formation of cellulose, hemicellulose, and lignin (Fan et al. 2006a). The lignin acts as an external crosslink binding hemicellulose and cellulose with cellulose positioned at the inner core of the structure (Fan et al. 2006a; Lin and Tanaka 2006). The lignocellulosic structure gives mechanical strength to plant cell walls and makes it naturally resistant to the microbial and enzymatic degradation (Figure 2.1) (Fan et al. 2006a). This resistance is generally known as "biomass recalcitrance" (Lin and Tanaka 2006). It is this characteristic that is mainly the reason for the high cost of lignocellulosic conversion (Fan et al. 2006a; Lin and Tanaka 2006). The physical, chemical, biological and thermal processes of biomass conversion yield many products. Biomass can either be used directly for combustion and co-firing for home heating, providing process heat for industrial facilities, generation of electricity, or application as liquid and gas fuels in the form of bioethanol or biogas, respectively, as well as a source of variety of bioproducts (Demirbas 2001). Such bioproducts generated from biomass can also be used as tools to enhance economic security. The growth of the bioenergy industry is closely tied to the availability of the technological path taken in the conversion of biomass-to cellulosic ethanol (Demirbas 2001).

In general, lignocellulosic biomass-to-cellulosic ethanol conversion processes includes: (a) initial particle size reduction; (b) pretreatment; (c) densification (optional, depending if the feedstock need to be transported); (d) enzymatic/acid hydrolysis/saccharification; (e) fermentation; and finally, (f) distillation process.

2.3.2 Chemical Structure and Composition

The quantity and ratio of each chemical constituent varies depending on the type of LB. Based on mass percentage, cellulose and hemicelluloses are higher in hardwoods compared to softwoods and crop straws; the lignin content of softwoods is generally higher than that of

hardwoods (Fan et al. 2006a; Lin and Tanaka 2006). Straws have a high percentage of extractives, which will be discussed later in this section. Lignin is the binding agent that gives plants rigidity and holds it together (Lin and Tanaka 2006). Hemicellulose and cellulose are complex carbohydrates bound within the cells of the plants, and these substances have the potential for bioethanol production (Fan et al. 2006a).

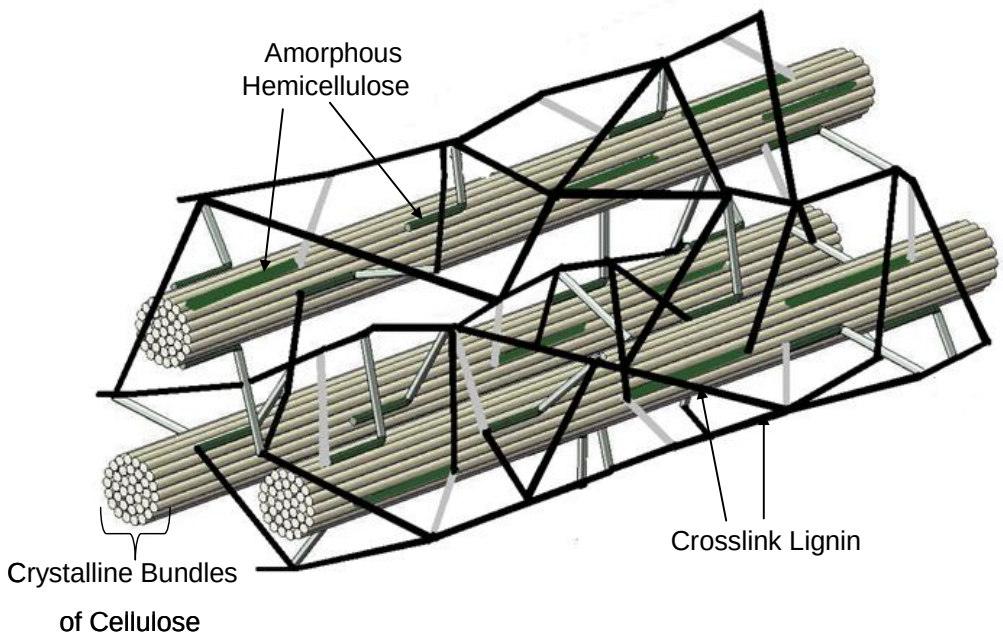


Figure 2.1 The general structure of lignocellulosic biomass (adapted from Shleser 1994; Murphy and McCarthy 2005; Shaw 2008).

2.3.2.1 Cellulose

Fan et al. (2006a) reported that cellulose content varies between species in the range of 40-50%, and it is the most surplus source of carbon in biomass and on earth. Cellulose is a skeletal polysaccharide that is widely distributed in the cell wall component of wood, agricultural, and municipal cellulosic waste. It exists as an un-branched chain, a fibrous, tough, water-insoluble compound present in the cell walls of plants (Fan et al. 2006a; Lin and Tanaka 2006; Ramesh and Singh 1993). Cellulose is a linear homopolymer of D-glucose residues linked by β-1,4-glycosidic bonds. This linearity leads to an orderly arranged cellulose chains that connect through the inter-molecular and intra-molecular hydrogen bonds comprising of hydroxyl groups

and hydrogen atoms of nearby glucose units (Figure 2.2) (Ramesh and Singh 1993). Hence, cellulose exists generally as crystalline fibers with minor presence of amorphous regions. The proportion of crystalline fraction ranges between 50 to 90% (Ramesh and Singh, 1993). This crystallinity nature of cellulose fibers is a major obstacle for efficient enzymatic saccharification/hydrolysis. Also, the largely lignified cell wall that surrounds the cellulose fiber reveals the cementing contribution of lignin and hinders the hydrolysis of cellulose (Fan et al. 2006a). Fan et al. (2006a; 2006b) reported that the hydrolytic enzyme attack on cellulose depends on its structural features, which includes: the surface area, the crystallinity of the cellulose, degree of polymerization, and the lignin seal surrounding the cellulose fibers, which leads to the structural resistance of cellulose (Jeoh et al. 2007). For the enzyme activity to proceed during hydrolysis, the interaction between the enzyme molecules and the surface of cellulose particles need to be well established.

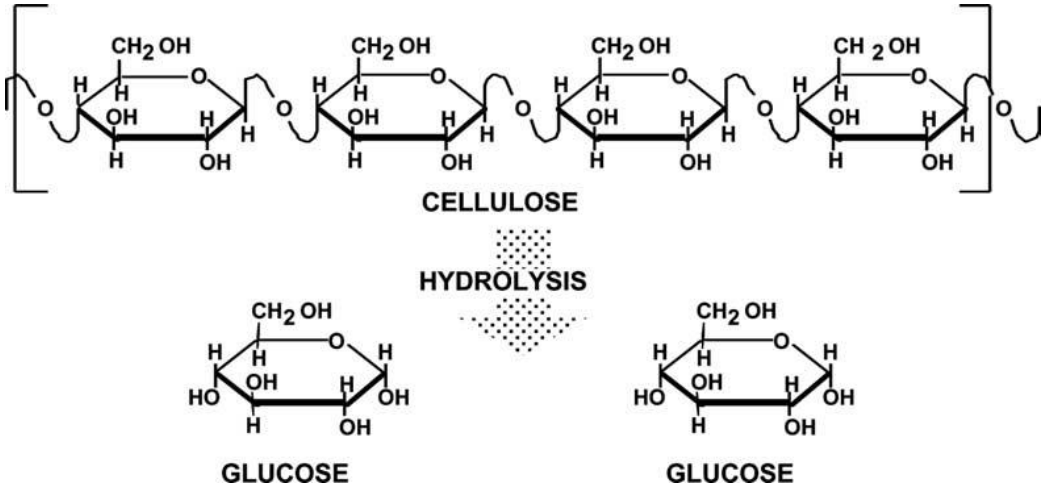


Figure 2.2 Structure of cellulose hydrolysis (adapted from Shleser 1994; Murphy and McCarthy 2005).

2.3.2.2 Hemicellulose

Compared to cellulose, hemicellulose is a highly branched heteropolymer, consisting primarily of five- and six-carbon sugars. It makes up 20-40% of the dry weight of LB (Ramesh and Singh 1993). It is composed of three hexoses: glucose, mannose, and galactose, and two pentoses: xylose and arabinose. The main hemicellulose is xylose, which consists of a xylan as the main

chain (backbone) made up of β-1, 4-linked D-xylose units with the other groups mentioned above as branches (Ramesh and Singh 1993). The presence of the side chains reduces the hydrogen bonding. Therefore, hemicellulose has lower crystallinity and higher amorphous structure which is more easily hydrolyzed unlike cellulose (Jeoh et al. 2007). The C5 and C6 sugars are linked through glycosidic bonds, forming a loose and very hydrophilic structure. Hemicellulose is chemically bonded to lignin and it serves as an interface between the lignin and the cellulose (Ramesh and Singh 1993; Jeoh et al. 2007).

2.3.2.3 Lignin and its Role in Enzymatic Hydrolysis

Lignin has a high molecular weight structure and it is a cross-linked complex aromatic macromolecule (Fan et al. 2006a). It is hydrophobic and characterized by high resistant to chemical and biological degradation (Ramesh and Singh 1993). It acts as cement among the plant cells, and in the different sections of the cell wall. It provides the structural rigidity to plants, forming cementing matrix covering hemicellulose and cellulose fibrils which further protect the entire matrix against biodegradation (Fan et al. 2006a). Lignin content and composition differs among different plant units (Ramesh and Singh 1993; Fan et al. 2006b). It contains about 50% more carbon relative to cellulose. It is present in cell walls of all vascular plants. The spaces among cellulose, hemicellulose and pectin are filled with lignin, therefore, it acts as binder among cell wall components (Ramesh and Singh 1993). Fan et al. (2006a) and Fan et al. (2006b) reported that the mixture of lignin and the crystalline cellulose produces one of nature's most biologically resistant material. As a result, microorganisms find it very difficult to degrade the cellulose because of the covalent chemical bonds and hydrogen bonding that exists between the constituents. Lignin is a three-dimensional polyphenolic network that comprises dimethoxylated (syringyl), monomethoxylated (guaiacyl) and non-methoxylated (p-hydroxyphenil) phenylpropanoid units, obtained from the corresponding p-hydroxycinnamyl alcohols, resulting to different sub-units, including various ether and C-C bonds (Fan et al. 2006a; Ramesh and Singh 1993). This three dimensional networks of lignin surrounding the cellulose hinder easy accessibility to enzymatic degradation. It makes up 10-25% of the dry weight of biomass (Fan et al. 2006a; Ramesh and Singh 1993; Fan et al. 2006b). The composition of different lignocellulosic material is presented in Table 2.2. The separately

recovered lignin after fermentation can be used for bioproducts and co-production of power and heat. Figure 2.3 shows a biomass-to-ethanol flowchart:

Table 2.2 Cellulose, hemicellulose and lignin content of different lignocellulosic biomass

Lignocellulosic Materials	Cellulose	Hemicellulose	Lignin	References
		(%)		
Barley straw	40	20	15	a
Coastal bermudagrass	25	35.7	6.4	b
Corn cobs	45	35	15	b
Corn stalks	35	15	19	a
Cotton seed hairs	80-95	5-20	0	b
Grasses	25-40	35-50	10-30	b
Leaves	15-20	80-85	0	
Newspaper	40-55	25-40	18-30	
Nut shells	25-30	25-30	30-40	b
Oat straw	41	16	11	a
Paper	85-99	0	0-15	
Primary wastewater solids	8-15	NA	NA	
Rice hulls	36	15	20	a
Rice straw	32	24	13	a
Saw dust	55	14	21	a
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7	
Sorghum straw	33	18	15	a
Sorted refuse	60	20	20	
Swine waste	6.0	28	NA	
Switchgrass	45	31.4	12	b
Wheat straw	30	50	15	b
Waste papers from chemical pulps	60-70	10-20	5-10	
Hardwood:				c
Birch	38.2	19.7	22.4	
Willow	43.0	29.3	24.2	
Softwood:				c
Pine	46.4	22.9	29.4	
Spruce	43.4	18	28.1	

(Ramesh and Singh 1993 (a); Jørgensen et al. 2007 (b); Kumar et al 2009 (c)).

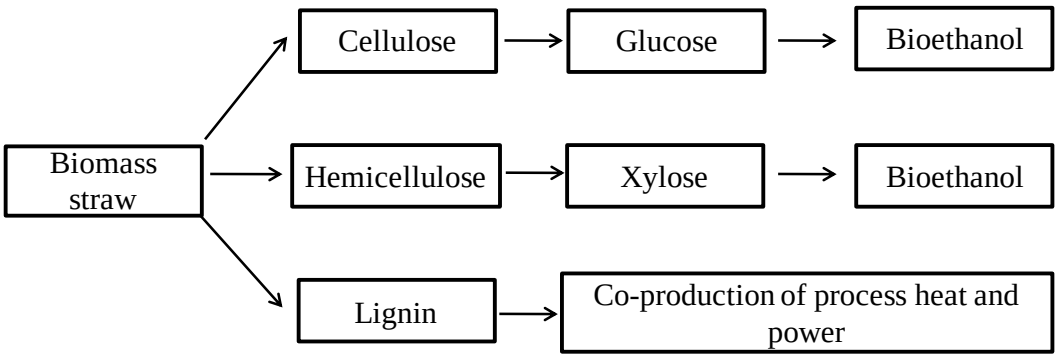


Figure 2.3 Lignocellulosic biomass-to-ethanol flowchart.

2.3.2.4 Extraneous Materials

In addition to the three main components, LB also contain some extraneous compounds with smaller molecular size, and available in little quantity. These include extractives and non-extractives. Agricultural residues contain little more above 8% extractives on dry weight basis (Ramesh and Singh 1993). These are waxes, fats, gums, starches, alkaloids, resins, tannins, essential oils, and many other cytoplasmic constituents. Some extractives are toxic and this is an advantage of wood to resist attack by fungi and termites. The non-extractives in agricultural biomass residues can be approximately 10% of the dry weight, which includes inorganic compounds (silica, carbonates, oxalates, etc) (Ramesh and Singh 1993).

2.4 Pretreatment of Lignocellulosic Biomass

Pretreatment is generally aimed at breaking down the lignocellulosic matrix, loosening the highly crystalline structure of cellulose and shifting it to the amorphous state. It also increases the porosity of the biomass (Sun and Cheng 2002). The cost of bioethanol production from LB is relatively high considering the available current technologies, and the major hurdles are the low conversion yield and high cost of the hydrolysis process (Sun and Cheng 2002). Considerable improvement has been made in the area of research to enhance the hydrolysis of LB. Therefore, for the realization of the biofuel production potentials of LB, attention needs to given to the existing challenges. The enzyme-based route of hydrolysis involves biomass pretreatment, because LB is structured for resistance and recalcitrance to biological, physical, and chemical attack (Söderström et al. 2003). There is usually low yield when non-treated/native LB is

subjected to enzymatic hydrolysis due to the high stability of the material to enzymatic or bacterial attacks (Söderström et al. 2003). Fan et al. (2006b) cited that most often, biodegradation of non-treated LB results in low yield of sugar from enzymatic hydrolysis, generally below 20% of the theoretical maximum.

The association of lignin with cellulose in LB makes the enzymatic hydrolysis of cellulose hard to deal with and laborious (Fan et al. 2006b). To achieve high conversion rate, the enzymatic process then requires relatively high enzyme loadings (high cost) to produce monomeric carbohydrates that are readily fermentable by microorganisms (Fan et al. 2006b; Sun and Cheng 2002; Söderström et al. 2003). This low rate of conversion inhibits the establishment of an economically feasible hydrolytic process. Hence, it is necessary to perform a pretreatment process before the enzymatic hydrolysis so as to significantly enhance the accessibility of the cellulosic material and adjustment of the organized structural arrangement in the LB, as shown in Figure 2.4. Furthermore, for logistics purposes, the loose and low bulk density nature of LB requires densification. Most LB species (straws and stover) have difficulty of forming or extrusion during densification in the absence of artificial and expensive binders (Sokhansanj et al. 2005). Past research shows that modifying the structural arrangement of cellulose-hemicellulose-lignin matrix can improve the natural binding features of the lignocellulosic straw and stover biomass (Sokhansanj et al. 2005). This implies that biomass has its own natural binder which can be harnessed through pretreatment process. Therefore, pretreatment of biomass makes it suitable for densification. Densification of biomass will be discussed in a later section.

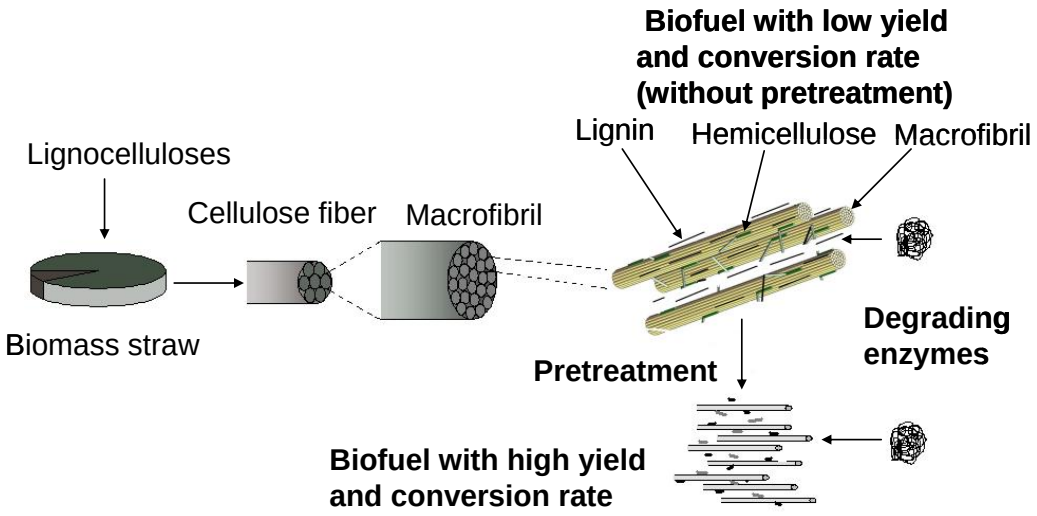


Figure 2.4 Effect of pretreatment on accessibility or biodegradability of lignocellulosic biomass (adapted from Mohammad and Karimi 2008).

A successful and cost-efficient pretreatment should satisfy the following requirements: (a) making the cellulosic fiber accessible for enzymatic activity; (b) hemicelluloses and cellulose should not be damaged; (c) prevention of the formation of substances that inhibits the subsequent activities of hydrolytic enzymes and fermenting microorganisms; (d) minimize loss of carbohydrates; (e) minimize the energy demand and the cost of pretreatment; (f) generating less residues; and (g) low operation cost such as the cost of labor, reactors, chemicals and enzymes (Kumar et al. 2009; Mohammad and Karimi 2008).

A survey of literature shows that several pretreatment methods has been investigated in recent time. Pretreatment methods are largely classified into physical, physico-chemical, chemical, and biological based on their main effects on the substrate (Mohammad and Karimi 2008). Detailed explanations of the various processes have been presented by Fan et al (2006b), Sun and Cheng (2002), Mohammad and Karimi (2008), and Mosier et al. (2005). Table 2.3 shows the classification of these pretreatment processes.

Table 2.3 Pretreatment schemes of lignocellulosic biomass (Adapted from Fan et al. 2006b; Mohammad and Karimi 2008.

Physical	Physicochemical & Chemical	Biological
Milling: - Ball milling - Two-roll milling - Hammer milling - Colloid milling - Vibro energy milling **Irradiation:** - Gamma-ray irradiation - Electron-beam irradiation - Microwave irradiation **Others:** - Hydrothermal - High pressure steaming - Expansion - Extrusion - Pyrolysis	**Acid**: - Sulfuric acid - Hydrochloric acid - Phosphoric acid **Alkali:** - Sodium hydroxide - Ammonia - Ammonium Sulfite **Explosion:** - Steam explosion - Ammonia fiber explosion (AFEX) - CO_2 explosion - SO_2 explosion **Gas:** - Chlorine dioxide - Nitrogen dioxide - Sulfur dioxide **Oxidizing agents:** - Hydrogen peroxide - Wet oxidation - Ozone **Solvent extraction of lignin:** - Ethanol-water extraction - Benzene-water extraction - Ethylene glycol extraction - Butanol-water extraction - Swelling agents	Fungi and actinomycete

2.4.1 Physical Pretreatment

Physical pretreatment of lignocelluloses basically involves particle size reduction such as grinding, milling or chopping. In general, it is divided into two categories; mechanical and non-mechanical methods (Fan et al. 2006b). For the mechanical pretreatments, physical forces are used to fractionate the LB into fine particles. Particle size reduction increases the accessible surface area of biomass particles and facilitates inter-particle bonding. It alters the degree of crystallinity and polymerization of the cellulose (Fan et al. 2006b; Mohammad and Karimi 2008). This makes the bulk of the material to be easily degraded by enzymatic hydrolysis, resulting from the increased surface-volume ratio (Fan et al. 2006b). The size of the material is usually 10-30 mm after chopping and 0.2-2 mm after milling or grinding (Kumar et al. 2009). The energy requirement for mechanical comminution of biomass materials is dependent on the final screen size of the biomass particle and characteristics (Kumar et al. 2009). In most cases, high energy consumption is needed to realize high yield of enzymatic hydrolysis. This may be sometimes higher than the theoretical energy content present in the biomass material (Kumar et al. 2009). Non-mechanical physical pretreatments basically expose the material to severe external forces. Such methods include irradiation, high pressure steaming, and pyrolysis (Fan et al. 2006b). Different types of physical pretreatment processes are use to improve the biodegradability of LB or enzymatic hydrolysis. Some selected types of physical pretreatment are briefly discussed in this section:

2.4.1.1 Ball Milling

This involves the application of shearing and compressive forces of the ball on the LB. It is an effective method of size reduction because it converts cellulose from crystalline to amorphous form, decrease the mean degree of polymerization of cellulose and hemicellulose, and increase the bulk density, thus, enhancing the enzymatic hydrolysis of LB (Fan et al. 2006b). Ball milling, as a form of physical pretreatment, has been successfully applied on cellulosic materials to enhance enzymatic hydrolysis (Mais et al. 2002). Mais and co-workers (2002) concluded that the higher the number of ball beads used in the controlled reaction volume during mechanical milling, the higher the rate of enzymatic hydrolysis of α-cellulose with a minimum enzyme loading. Fan et al. (2006b) cited that the rate of hydrolysis conversion is a function of the ball

milling time. Ball milling effectiveness varies from material to material, with soft woods (pine, spruce) showing the least response, and hard woods (aspen, birch) showing the best response. This makes this method of physical pretreatment not a generally acceptable one (Fan et al. 2006b)

2.4.1.2 Two Roll Milling

The set up consists of two cast-iron tempered surface rolls, positioned horizontally; the gap between the rolls can be adjusted by screws (Fan et al. 2006b). Recently, this method has been applied on LB to enhance the digestibility and accessibility. It reduces the crystallinity and the degree of polymerization of polysaccharides; its effects on lignin are not well understood in the scientific community (Fan et al. 2006b). Fan et al. (2006b) cited that the clearance between the roll mills and the duration of milling are the two parameters that control the accessibility of enzymatic attack. Fan et al. (2006b) reported that there was a considerable decrease in the crystallinity index and a drastic increase in the accessibility of cellulose after compression milling.

2.4.1.3 Extrusion and Expansion

These have similar features as the high pressure steaming. This pretreatment involves moist-heat expansion (extrusion) and dry-heat expansion (popping). They are used to increase feed efficiency of cereal grains in animal feeds (Fan et al. 2006b). Fan et al. (200b) cited that the application of extrusion pretreatment is not effective in improving the digestibility and degradability of rice straw and sugarcane bagasse.

2.4.1.4 High Energy Radiation

The accessibility and digestibility of LB can be improved by the application of high energy radiation such as gamma rays, electron beam, and microwaves (Mohammad and Karimi 2008; Millett et al. 1970). This process results in dehydrogenation, oxidative degradation, destruction of anhydroglucose units to produce carbon dioxide, and cellulosic change cleavage (Millett et al. 1970). Beardmore et al. (1980) reported that gamma radiation of solka floc increases the surface area due to extensive depolymerization. However, it was concluded that this method is not

effective in converting the crystalline to amorphous form of cellulose. Fan et al. (2006b) reported that the optimum accessibility and digestibility of wheat straw was realized at 2.5 x 10^8 rads using gamma radiation. Digestibility can be improved by subjecting the material to milling followed by addition of nitrate salts before irradiation, or alternatively, saturate the environment with oxygen during irradiation (Florine and Arthur 1958). The high cost of this method of physical pretreatment has limited its applicability.

2.5 Physico-Chemical Pretreatment

Chandra et al. (2007) reported that the combination of chemical and physical processes of pretreatment is called physico-chemical processes. The most important methods of this process will be discussed in this section:

2.5.1 Steam Explosion (Autohydrolysis)

The idea of deconstruction of LB necessitated the use of pretreatment in pulp and paper manufacturing (Chornet and Overend 1988). This method has recently received considerable attention in the pretreatment of LB for the production of both bioethanol and biogas. It may involve steaming with or without explosion (autohydrolysis) (Mohammad and Karimi 2008). In steam explosion process, the pressure is suddenly released, causing the LB to encounter an explosive decompression (Kumar et al. 2009). It involves fractionation of LB into its major components (hemicelluloses, cellulose, lignin, and extractives) (Brecc and Glasser 1998). Most of the recalcitrance of LB is removed through this process, hence, increasing the degree of accessibility and digestibility of the feedstock. Montane et al. (1998) applied steam explosion as a pretreatment method on wheat straw using high pressure and high temperature between 205 and 230^{o}C and a reaction time of 2 minutes. These researchers concluded steam explosion to be an effective pretreatment process for the fractionation of wheat straw into its constitutive polymers. During the pretreatment process, increasing the temperature up to a certain amount (190-210^{o}C) can adequately release hemicellulosic sugars. However, the sugar degradation readily increases with further increase in the temperature (220-230^{o}C), which results to decrease in total sugar recovery (Mohammad and Karimi 2008). Steam explosion pretreatment process is

an interesting option for LB in an ethanol production scheme. Some researchers have demonstrated the advantage of adding sulfur dioxide (SO_2) to LB prior to pretreatment. The aim is to improve the recovery of both cellulose and hemicellulose fractions. The highest overall conversion yield of glucose and xylose was achieved at optimum conditions for one-step steam explosion pretreatment of LB (corn stover and willow wood chips) for fuel ethanol production, using SO_2 as a catalyst at temperatures between 180-210°C with residence time of 4-7 minutes (Chornet and Overend 1988; Öhgren et al. 2005; Sassner et al. 2005).

Several other research groups such as Rai and Mudgal (1987) and Bress and Glasser (1998) to mention but a few, have found steam explosion process as a good pretreatment process. The energy cost is comparatively moderate, and it meets most requirements of the pretreatment process. Sun et al. (2005a) pointed out that special consideration should be taken in choosing the steam explosion operating conditions so as to avoid excessive biodegradation of cellulose resulting in changes of its physical and chemical properties. They reported that in a very severe condition, lesser enzymatic digestibility of LB may also be detected after steam explosion. Like in the case where there is generation of condensation or inhibitory substances (furfurals) from the polymers (cellulose and hemicellulose) in steam explosion of wheat straw that may result to a more non-degradable residues. Therefore, it is necessary to wash with water to remove part of these inhibitory substances; however, washing with water increases the amount of wastewater from the pretreatment process (Chundawat et al. 2006).

2.5.1.1 Mechanism of Steam Explosion

Steam/aqueous explosion is a thermomechanochemical process. It involves the breakdown of structural components which is assisted by heat in the form of steam (thermal process), occurrence of shear forces resulting from the expansion of moisture (mechanical process), and finally hydrolysis of glycosidic bonds/linkages of hemicellulose and alkyl-aryl linkages of lignin to produce low molecular weight fragments (chemical process) as described by Chornet and Overend (1988) and Tanahashi et al. (1982).

Chornet and Overend (1988) analyzed the process of steam explosion in a reactor, under high pressure, as follows: the steam penetrates the lignocellulosic structures first via diffusion. The penetration of steam into the inner structures of the LB is permitted because vapor phase diffusion is higher than the liquid phase diffusion. At high pressure, steam condenses, and subsequently wets the "capillary-like" microporous structure of LB. Hydrolysis takes place if the temperature is high enough to thermodynamically initiate the breakdown of liquid water and thus build up an acidic medium, which subsequently overcome the energy barriers for hydrolysis (Chornet and Overend 1988). The condensed moisture hydrolyzes the acetyl units of the hemicellulose portions to form organic acids such as acetic and uronic acids. These acids act as catalysts in the depolymerization of hemicellulose, yielding xylan and restricted quantity of glucan (Chornet and Overend 1988). At severe conditions, the amorphous portion of cellulose may be hydrolyzed to a certain degree. However, at extreme conditions (at high temperatures and pressures), degradation of xylose to furfural and glucose to 5-hydroxymethyl furfural occurs. Furfural is an undesirable compound in fermentation because it inhibits microbial growth and activity (Chornet and Overend 1988). After the required residence time, the "wet" biomass is "exploded" upon the sudden release of the pressure within the reactor. The "wetted"/"treated" biomass is driven at high velocities and ejected out of the reactor via a small nozzle by the induced force (sudden opening of the discharge valve, i.e. blow down) (Chornet and Overend 1988). Chornet and Overend (1988) further reported that as a result of the sudden decrease in pressure, the following changes occur:

- instantaneous cooling due to evaporation of the condensed moisture within the lignocellulosic structure
- evaporation of water and expansion of the water vapor generates and exerts shear forces on the surrounding "cylindrical tubes" structure known as fibers. If the shear force is high enough, the vapor will result in the mechanical dissociation of the lignocellulosic structure.

Chornet and Overend (1988) and Wang et al. (2009) reported that the retention time and temperature are the process parameters required for the optimization of the process. Hydrolysis of hemicellulose is of great importance to the downstream fermentation process. The degree of hydrolysis of hemicellulose by the organic acids is determined by the length of time the biomass

stays in the reactor and the operating temperature of the process. However, long retention time increases the production of inhibitory substances, which must be minimized (Chornet and Overend 1988; Wang et al. 2009).

The operating temperature controls the steam pressure within the reactor. The water contained within the capillaries (before discharge) at high temperature is in equilibrium with the high pressure in the reactor. Higher temperatures lead to corresponding higher pressures, which subsequently increases the difference between the reactor and atmospheric pressure. The pressure difference is equal to the induced shear force of the evaporating moisture (Chornet and Overend 1988).

2.5.1.2 Severity Factor

Due to the different types of available LB, severity factor has been developed to standardize the process parameters of steam explosion so as to facilitate comparisons. It relates the net product yields and pretreatment severity (Chornet and Overend 1988). Effort has been in progress to minimize product degradation resulting from pretreatment conditions. This model according to Chornet and Overend (1988) defines the severity factor of a steam explosion pretreatment with respect to the combined effect of temperature and the retention time. The model was developed based on the postulation that the process kinetics is first-order, which follows the Arrhenius' model (Chornet and Overend 1988):

$$k=Ae^{-Ea/RT} \quad (2.2)$$

k = rate constant

A = Arrhenius frequency factor

Ea = activation energy (kJ/kg mol)

R = universal gas constant (8.314 kJ/kg mol K)

T = absolute temperature (K)

The reaction ordinate was developed as:

$$R_o = \int_0^t \exp\left[\left(T_r - T_b\right)/14.75\right] dt \quad (2.3)$$

R_o = reaction ordinate

t = residence time (min)

T_r = reaction temperature (^oC)

T_b = base temperature at 100^oC

(14.75 is the conventional energy of activation postulating that the overall process is hydrolytic and the overall conversion is first order) (Chornet and Overend 1988).

Therefore,

$$R_o = t*\exp[(T_r - 100)/14.75] \quad (2.4)$$

The log value of the reaction ordinate yields the severity factor which is used to analyze the effects of steam explosion pretreatment on LB.

$$\text{Severity} = \log_{10}(R_o) \quad (2.5)$$

Heitz et al. (1991) reported that it is possible to directly translate results obtained from laboratory steam explosion pretreatment process into industrial practice by employing the concept of a severity factor. This is similar to those used in pulping industry which integrates temperature and the retention time into a single measure for the purpose of controlling the process (Tanahashi et al. 1982).

Heitz et al. (1991) successfully modeled the recovery trends of lignocellulosic polymers from steam explosion of *Populus tremuloides* as a function of the severity factor, by applying high pressure steam at temperatures between the range of 180-230^oC and retention time of 0.7-4 min. It was demonstrated that the higher the operating conditions (temperature and time), the higher was the severity parameter (R_o), resulting in higher recovery of cellulose and lignin, which enhances the accessibility and digestibility of cellulose. However, they stated that this recovery

is at the expense of increasing the destruction and degradation of hemicellulose. It was suggested that the optimum treatment severity of each of the polymer fractions would probably involve a two-stage treatment cycle, which will require the removal of the bulk of the hemicellulose and lignin after the first stage at low severity, and second post-treatment stage to obtain high purity cellulose. However, this two-stage treatment cycle may result to low degree of polymerization (Heitz et al. 1991).

Kaar et al. (1998) investigated steam explosion of sugarcane bagasse as a pretreatment for conversion to bioethanol by applying severity factor employing a spectrum of operating temperatures (188-243^{o}C) and retention times (0.5-44 min) in a 10 liter batch reactor. The results indicated that the processing optimal conditions of steam explosion are highly biomass feedstock dependent, because of the varying carbohydrates composition of LB feedstocks. Biomass feedstocks high in xylose content (hemicellulose) require moderate process conditions, with lesser experimental run times, relative to feedstocks lower in xylose but higher in glucose (cellulose) content Kaar et al. (1998). These researchers observed that inhibitor compound (e.g. furfural) was produced due to the extreme treatment condition of hemicellulose. It was concluded that the validity of the reaction ordinate approach does not apply universally. However, the reaction ordinate remains all-purpose bookkeeping technique of reporting steam explosion conditions Kaar et al. (1998).

2.5.1.3 Physical and Chemical Effects of Steam Explosion Pretreatment on Lignocellulosic Biomass

The steam explosion process is characterized by a physical rupture of the LB structure into fine fibers and/or powder by adiabatic expansion of water in small pores in LB tissues, and autohydrolysis of the cell components (Tanahashi et al. 1982). Tanahashi et al. (1982) investigated the effects of steam explosion on the morphological structure and physical properties of LB using shirakanba and karamatsu wood chips. The study demonstrated that the degree of crystallinity and width of micelle increased by about 1.5 and 2.0 times, respectively, as a result of the treatment, which indicates that most of the amorphous portion of cellulose are converted to crystalline region at higher steam pressure, leading to a rise in the "degree of

crystallinity and micelle width" of the exploded LB. At moderate severities, steam explosion promotes good delignification, which is useful for the preparation of cellulose microfibrils and lignin for the subsequent stage of the bioethanol production (Tanahashi et al. 1982). Tanahashi et al. (1988) studied the chemical effects of steam explosion on wood. It was observed that during steaming at lower severities (1,962 kPa, 1 min), hemicellulose fractions were being readily hydrolyzed to oligosaccharides. At higher severity conditions, hemicellulose further hydrolyzed to monosaccharides. However, at this severity, the concentration of furfural and 5-hydroxymethyl furfural increased.

X-ray diffractograms of steam exploded poplar feedstocks demonstrated that higher severity treatment conditions led to increasing pattern of the cellulose network structure, thus increasing crystallinity (Atalla 1988). Excoffier et al. (1988) reported that the extent of crystallinity of cellulose increased as a result of the steam explosion treatment condition. These researchers attributed this findings to the crystallization of amorphous portions of cellulose during steam explosion treatment process. It was also indicated that hydrolysis and depolymerization of hemicellulose and lignin, respectively, occurred during the steam explosion process. It should be noted that lignin has a softening temperature between 130-190^{o}C (Fengel and Wegener 1984).

2.5.2 Ammonia Fiber Explosion (AFEX)

This process involves the explosion of the biomass feedstocks at a relatively low temperature (compared to steam explosion) so as to prevent sugar decomposition (Alizadeh et al. 2005). AFEX is an alkaline physico-chemical pretreatment mechanism. The LB is placed in liquid ammonia at temperature range of 90-100^{o}C for a time duration of about 5-30 min, followed by a decrease in pressure (Alizadeh et al. 2005). The required parameters in the AFEX technique are ammonia and water loading, time, temperature, and blow down pressure (Alizadeh et al. 2005; Teymouri et al. 2004). The yield of this pretreatment method is only a pretreated solid material, unlike some other pretreatments methods (for example steam explosion) that generate slurry which can be separated into fractions of solid and liquid (Mosier et al. 2005). The lignin present in the LB can be effectively modified while the cellulose and hemicellulose portions may remain unbroken. The AFEX process can substantially enhance the enzymatic hydrolysis if the optimum

conditions are established. The optimum conditions for AFEX are dependent on the LB (Alizadeh et al. 2005). Teymouri et al. (2004) studied the effect of pretreatment using AFEX on corn stover where the highest glucan and xylan conversions and ethanol yields from AFEX-treated corn stover were achieved. It was pointed out that enzymatic hydrolysis of corn stover treated under optimal AFEX conditions (90^{o}C, 60% (dry weight basis), 5 min, and ammonia-corn stover mass ratio of 1:1) yields almost 98% glucan conversion and 80% xylan conversion against 29 and 16% for untreated corn stover, respectively (Teymouri et al. 2004). This method of pretreatment has one major advantage; it does not have formation inhibitors as by-products/sugar monomers, unlike some pretreatment methods (steam explosion and dilute-acid pretreatments) that produce such inhibitors, such as furans (Teymouri et al. 2004).

However, there are some disadvantages associated with the AFEX pretreatment method. It is more efficient on the LB that has small amount of lignin, it does not substantially solubilize hemicellulose unlike other types of pretreatment processes (e.g. dilute-acid pretreatment) (Teymouri et al. 2004). Finally, it involves a difficult recycling of the feed ammonia as a reusable gas stream after the pretreatment so as to decrease the pretreatment cost and to keep the environment safe at the same time (Sun and Cheng 2002; Wyman 1996). Since ammonia is expensive, not being able to totally reuse has a negative effects to the process economy (Zheng et al. 1995). Furthermore, the corrosive and toxic nature of ammonia may result to design and operation difficulty (Sun and Cheng 2002; Teymouri et al. 2004; Wyman 1996).

2.5.3 Supercritical CO_2 Explosion (SC- CO_2)

This method has been mainly used for solvent extraction. SC-CO_2 explosion has been considered to be used for non-extractive purposes as a result of its several advantages (Kim and Hong 2001). CO_2 is available at low cost; it is clean and environmentally friendly, and it is not difficult to recover after use (Kim and Hong 2001). To reduce the cost observed in the ammonia explosion and to avoid sugar degradation (such as those due to steam explosion) that results from high temperature, the SC-CO_2 explosion seems to be an alternative, because of its low operating temperature. Kim and Hong (2001) and Zheng and Tsao (1996) have demonstrated that the use of SC-CO_2 as a pretreatment can effectively improve the accessibility of cellulose by enzymatic

hydrolysis to produce glucose. SC-CO_2 explosion pretreatment method improves the degradation of LB upon a sudden explosive release of CO_2 pressure. This process increases the accessible surface area of the cellulosic substrate to enzymatic hydrolysis (Zheng et al. 1995). It was concluded that the higher the pressure of CO_2, the higher was the glucose yield, an indication that CO_2 molecules penetrate faster into the cellulose pores at higher pressure (Zheng et al. 1995).

There are several attractive benefits of using this method, these includes: non-flammability, non-toxicity, readily available at low cost, and environmental acceptability. It is highly applicable in the biochemical and pharmaceutical industries. Simultaneous application of SC-CO_2 explosion pretreatment and enzymatic hydrolysis in one stage has also been carried out to obtain 100% glucose yield (Park et al. 2001). However, for the application of this method, the high yield of sugar depends on the type of LB. It has a low pretreatment effectiveness for softwood. The capital intensive nature of high-pressure equipment may be an obstacle to the applicability and commercialization of this pretreatment method (Kim and Hong 2001).

2.5.4 Microwave-Chemical Pretreatment

Microwaves fill up a transitional portion in the electromagnetic (EM) spectrum between radio-frequency and infrared radiation. This portion of EM corresponds to a frequency range of 300 MHz to 300 GHz, with higher frequency range used for telecommunication and radar transmissions (Ryynanen 1995). To avoid interference, almost all domestic and industrial microwaves operate typically either at 900 MHz or 2.45 GHz (Punidadas et al. 2003; Ryynanen 1995). Microwave (MW) is part of the EM spectrum that results in "heating of dielectric materials by induced molecular vibration as a result of dipole rotation or ionic polarization" (Ramaswamy and Tang 2008). The heating process is based on volumetric heat generation. This implies that heat transfer is from inward to outward unlike the conventional heating system. MW ovens can also be used for commercial purposes include rapid extraction, blanching, drying, sterilization, pasteurization, selective heating, disinfestations, tempering, enhanced reaction kinetics, among others (Ramaswamy and Tang 2008). To widen the scope and science behind MW radiation, it has been used in the LB pretreatment. The microwave-chemical pretreatment is

a more efficient pretreatment method than the traditional heating chemical pretreatment due to the effective acceleration of the reactivity of cellulosic material (Zhu et al. 2005). Microwave radiation has been applied in different fields of science. It has been successfully applied in the fields of organic chemistry by Larhed et al. (2002) in accelerating organic transformations at a reduced reaction time. Several researchers have applied this method on LB and coming to the conclusion that it helps in breaking down lignin, while improving its accessibility and digestibility to hydrolytic enzymes. Zhu et al. (2006) investigated the effects of three different "microwave-chemical pretreatment processes (microwave-alkali, microwave-acid-alkali, and microwave-acid-alkali-H_2O_2)" on rice straw. The results shows that rice straw pretreated with microwave-acid-alkali-H_2O_2 had the highest rate of hydrolysis and glucose content in the hydrolysate. Donepudi and Muthukumarappan (2009) studied the effect of microwave power level and processing time with different levels of concentrations of acid and alkaline on the yield of glucose and xylose from corn stover. It was reported that increasing the microwave processing time from 0 to 5 minutes, led to a remarkable increase in glucose and xylose yields by 58.7 and 149.6%, respectively. The effect of microwave and alkali pretreatment on rice straw and its enzymatic hydrolysis was investigated by Zhu et al. (2005). The result was compared with alkali-alone pretreated process. The results indicated that rice straw subjected to microwave-alkali pretreatment had more glucose content in the hydrolysate and higher hydrolysis rate than the method that involved alkali pretreatment alone. Pretreatment with higher microwave power with shorter processing time and lower microwave power with longer pretreatment time has fairly similar effect on the weight loss and composition at the same energy consumption (Zhu et al. 2005). However, MW is known for non-uniform heat distribution, which could cause thermal runaway during the pretreatment process (Punidadas et al. 2003; Ryynanen 1995; Ramaswamy and Tang 2008).

2.6 Chemical Pretreatment

The "cellulose hemicellulose-lignin matrix can be broken down to smaller amorphous molecules through acid or alkaline hydrolysis as well as through" wet oxidation (Sun et al. 2004). Alkaline or acid solutions are frequently used for pretreatment of biomass materials. The effect of pretreatment depends on the lignin content present in the biomass material. Chemical

pretreatments are usually effective. However, they have disadvantages which should not be ignored. These include the use of specialized equipment that is corrosion resistant, the need for extensive washing, and the requirement for disposal of chemical wastes (Sun et al. 2004). Different chemical methods have been investigated, which includes the use of alkalis, acids, wet oxidation, gases, oxidizing agents, cellulose solvents, extraction, and swelling agents (Sun et al. 2004).

2.6.1 Acid Hydrolysis Pretreatment

Pretreatment with acid hydrolysis can lead to the enhancement of enzymatic hydrolysis of LB to release the fermentable simple sugars. H_2SO_4, HCL, H_3PO_4, and HNO_3 have been used to hydrolyze LB (Wyman 1996). Acid pretreatment can be performed either at low temperature (e.g. 40°C) and high acid concentration (e.g. 30-70%) or at high temperature and low acid concentration (Wyman 1996; Sun et al. 2004). Dilute-acid hydrolysis has been successfully applied in the pretreatment of LB. Studies have revealed that sulfuric acid below 4% (w/w) concentrations is usually the most interesting condition to effectively pretreat at low cost (Brecc and Glasser 1998). Dilute acid (H_2SO_4) was used by Saha et al. (2005) for pretreatment of wheat straw at varied temperature. The enzymatic hydrolysis was investigated for the conversion of cellulose and hemicellulose to monomeric sugars. Higher yield of ethanol was obtained with a corresponding decrease in the fermentation time. "Optimization of dilute-acid (H_2SO_4) pretreatment of corn stover using a high-solids percolation reactor" was investigated by Zhu et al. (2005). They reported that the digestibility and accessibility was related to the degree of xylan removal. The effect of increasing the concentration of sulfuric acid and residence time of the pretreatment process is significantly higher for Bermuda grass than rye straw for ethanol production (Sun and Cheng 2005a). Concentrated acids are toxic, corrosive, and dangerous, hence corrosion resistant reactors are needed for the operation. There is also a need to recover the used concentrated acid after the hydrolysis process so as to make the process economically attractive. These factors (high investment and maintenance costs) lower the commercial interest of this process (Wyman 1996; Sun et al. 2004).

2.6.2 Wet Oxidation

This method has been used as a pretreatment for the deconstruction of lignocellulosic structure for the production of both bioethanol and biogas. In this process, LB is treated with air or oxygen and water at temperatures between 150 and 200°C for a period of about 10 min (Palonen et al. 2004). The temperature, reaction time, and oxygen pressure are the major factors in wet oxidation pretreatment (Palonen et al. 2004; Martin et al. 2008). Oxygen plays a very important role in the degradation reactions and initiates operation at relatively reduced temperatures by improving generation of organic acids (Taherzadeh and Karimi 2007). The main reactions "are the formation of acids from hydrolytic processes, as well as oxidative reactions". However, it is an exothermic process, with fast rates of reaction and heat generation, therefore, the control of reactor temperature is necessary (Palonen et al. 2004; Varga et al. 2004). Martin et al. (2008) investigated the potential of clover and ryegrass mixtures as feedstocks for the bioethanol production. Wet oxidation pretreatment at three levels of temperature (175, 185, 195ºC), 10 min residence time, and at two different oxygen pressures with or without the addition of sodium carbonate, was evaluated. The enzymatic hydrolysis of cellulose extremely increased without production of inhibitor. It was revealed that no addition of nutrients was required for the fermentation of clover-ryegrass hydrolysates (Martin et al. 2008).

2.6.3 Alkaline Hydrolysis Pretreatment

Sodium, potassium, calcium, and ammonium hydroxides are acceptable alkaline pretreatment agents (Kumar et al. 2009). This pretreatment method requires the use of some bases/alkaline solutions to remove lignin and a portion of the hemicellulose, and successfully improve the accessibility and digestibility of cellulose by enzymes (Jeoh et al. 2007; Mohammad and Karimi 2008). It enhances the saccharification process. Alkaline pretreatment can be done at low temperatures but at relatively long period of time with high concentration of the alkaline (Kumar et al. 2009; Mohammad and Karimi 2008). It has been applied as a pretreatment technique in biogas production (Mohammad and Karimi 2008). The effect of alkaline pretreatment is dependent on the lignin content of the biomass materials (Kumar et al. 2009; Kim and Holtzapple 2006). Alkaline processes result to lesser sugar degradation unlike the acid pretreatment. Most of the caustic salts from the process can be recovered and/or regenerated

(Kumar et al. 2009). Kumar et al. (2009) stated that "when biomass is treated with dilute alkaline solution, swelling occurs, which increases the internal surface area of the material. Swelling causes a decrease in the degree of polymerization, separation of structural linkages between lignin and carbohydrates, disruption of the lignin structure", and reduction of cellulose crystallinity (Kumar et al. 2009; Sun et al. 2002; Mosier et al. 2005). Among the four alkaline compounds mentioned, calcium hydroxide (slake lime) is accepted to be a suitable pretreatment agent, with the lowest cost per kilogram of hydroxide (Kumar et al. 2009). This is due to the possibility of recycling the "calcium from an aqueous reaction system as insoluble calcium carbonate, by neutralizing it with" low cost carbon dioxide; the calcium hydroxide thereafter can be regenerated using already discovered and well accepted lime kiln technology (Kumar et al. 2009). Alkaline pretreatment plays a very important role in making cellulose accessible to enzyme attack. The removal of lignin enhances the effectiveness of enzyme by removing non-productive adsorption sites, thereby, increasing the accessibility and digestibility to cellulose and hemicellulose (Kumar et al. 2009).

Kim et al. (2006) investigated corn stover by pretreating with excess calcium hydroxide (0.5 g $Ca(OH)_2$/g raw biomass) in non-oxidative and oxidative conditions. The enzymatic degradability and "digestibility of lime-treated corn stover was affected by the change of structural features such as acetylation, lignification, and" crystallinity resulting from the treatment. Soybean straw soaked in ammonia liquor (10%) for 24 hour at room temperature proved that this method is very effective in reducing the lignin by 30.16% (Xu et al. 2007). However, alkaline pretreatment indicated that it is more effective on agricultural biomass residues than on wood materials (Mohammad and Karimi 2008; Xu et al. 2007). Zhang et al. (2008) studied the pretreatment of rice straw using 2% NaOH to remove lignin. Their findings demonstrated that the pretreatment increased cellulose by 54.83% and decreased lignin by 36.24%, this could improve and facilitate the enzymatic hydrolysis process. Analysis of wheat straws indicated that hemicellulose, lignin, and silica were solubilized by NaOH pretreatment. The digestibility of different structural polysaccharides was higher for NaOH-pretreated straw than the native straw. However, at lower concentrations of alkaline solution, cellulose showed resistance to solubilization, but not at higher levels (above 7% w/w) (Zhang et al. 2008). Agricultural residues were treated at 100°C

for one to two hours with lime (CaO) to increase the rate and degree of dry matter digestibility. Gandi et al. (1997) concluded that lime treatment roughly doubled the digestibility making it an efficient technique to improve and upgrade the digestibility of agricultural biomass residues. Alkaline pretreatment leads to increase in ash content, which increases with increasing concentrations of the alkaline (Rai and Mudgal 1987).

2.7 Biological Pretreatment

Expensive instruments or high infrastructure cost with high energy consumption are used in most pretreatment technologies discussed previously. The physical and thermo-chemical pretreatment processes especially require excessive energy for biomass pretreatment. Biological pretreatment uses different types of lignin-degrading microorganisms (rot fungi) and it is a safe and environmentally benign method (Sun and Cheng 2002; Okano et al. 2005). This method of pretreatment is considered to be energy saving since it is performed at low temperature and requires no chemicals for lignin removal from the LB (Sun and Cheng 2002). Microorganisms such as brown-rot, white-rot, and soft-rot fungi are used to breakdown and degrade lignin and hemicellulose in biomass materials (Sun and Cheng 2002). Brown-rot fungi usually attack cellulose, while white- and soft-rot fungi attack both cellulose and lignin (Sun and Cheng, 2002). Carbon and nitrogen sources control the fungi reaction. White-rot fungi are the most efficient for biological pretreatment of LB (Sun and Cheng 2002; Okano et al. 2005; Hatakka 1983). Hatakka (1983) studied the biological pretreatment of wheat straw using some white-rot fungi (*Pleurotus ostreatus*, *Pleurotus* sp. 535, *Pycnoporus cinnabarinus*115, *Phanerochaete sordida* 37, *Phlebia radiata* 79 and *Ischnoderma benzoinum* 108) and reported that 35% of the straw was transform to reducing simple sugars by *Pleurotus ostreatus* in 5 weeks. However, the rate of biological pretreatment technique is relatively low for industrial application, and some materials are lost due to the consumption of hemicellulose and cellulose, or lignin to some extent by the microorganisms (Sun and Cheng 2002; Wyman 1996).

The summary of the merits and demerits of the various pretreatment methods on LB as discussed is shown in Table 2.4.

Table 2.4 Summary of merits and demerits of various processes used for the pretreatment of LB (adapted from Fan et al. (2006b) and Mohammad and Karimi (2008).

Pretreatment process	**Advantages**	**Limitations**
Mechanical comminution	Decreases cellulose crystallinity, increase in accessible surface area and pore size, decrease in degrees of polymerization, chemicals are generally not required for these process	Power consumption are generally higher than resident biomass energy
Steam explosion	Results lignin transformation; cost-effective, increase in accessible surface area, decrease in degrees of polymerization, usually rapid treatment rate, among the effective and promising techniques for industrial applications	Degradation of a portion of the xylan; incomplete disruption and breakdown of the lignin, carbohydrate matrix; generation of inhibiting compounds to microorganisms, chemical are required, typically need harsh conditions
Ammonia fiber explosion	Increases accessible surface area, eliminates lignin and hemicellulose to an extent; does not generate inhibitors for downstream processes, promising processes for industrial applications	Not efficient for biomass materials with high lignin content, there are chemical requirements
CO_2 explosion	Increases accessible surface area; no formation of inhibitory/toxic compounds. non-flammability, readily available at low cost, and environmental acceptability	High capital cost for high-pressure equipment, it depends on the type of LB
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other simple sugars; changes lignin structure.	High cost of corrosion resistant equipment; formation of inhibitory substances, there are chemical requirements
Alkaline hydrolysis	Lesser sugar degradation, removes lignin; increases accessible surface area, utilize lower temperatures and pressures, most caustic salts can be recovered and/or regenerated.	Long residence times required; there are chemical requirements, dependent on the lignin content of the LB
Biological	Degrades lignin; low energy requirements. no chemical requirement, environmental benign, decreases the degree of polymerization of cellulose	Rate of hydrolysis is very low, very low treatment rate, not considered for commercial application, degrades hemicelluloses

2.8 Densification of Biomass

In its natural form, most LB are bulky, loose and dispersed, hence they are difficult to utilize as fuel (Mani et al. 2006a). Hence, the large volume requirements of biomass as a feedstock for biorefinery does not present easy, economical, and efficient transportation, handling and storage characteristics (Tabil 1996; Mani et al. 2006a; Wooley et al. 1999). To mitigate this difficulty, pre-processing and densification of LB is offered as a solution. Biomass densification may be defined as the compaction or compression of biomass to remove inter- or intra-particle pore spaces. It also reduces the moisture content of biomass during compression. The main goal of densification is to increase mass per unit volume of the biomass (Granada et al. 2002).

A conventional method of pelleting/densification entails application of pressure and involves pressing biomass material through a long die (Mani et al. 2006a). The friction between the die wall and the material creates resistance to free flow of the material through the die. This resistance causes compression of the material if the die is tapered (Mani et al. 2006a). Mani et al. (2006a) cited that approximately 40% of the total applied energy was used for the compression of the biomass materials (straw and hay), while 60% was used to overcome friction. Therefore, a large amount of the applied energy for the densification of biomass is used to overcome friction. The same authors reported that the frictional energy can be decrease via preheating the feed or die surfaces, so as to maintain a smooth die surface, thereby reducing the time required to extrude the biomass. Pretreatment of biomass before densification would also considerably improve the compression property of the biomass material leading to lower energy consumption.

As summarized by Tabil (1996), upon the application of pressure, there are basically three main stages involved during the densification process: a) particle rearrangement; b) elastic and plastic deformation; and c) mechanical interlocking of particles and local melting of some constituents:

a) Particle rearrangement and breaking down the initial unstable packing arrangements is the first stage of densification, which is often called ‘arches’ or ‘bridges’. This stage happens at low pressures resulting to a closer packing.
b) Elastic and plastic deformation happens at higher pressures and involves two sub-stages that correspond to elastic and plastic deformation. Due to the higher pressures, the

particles are forced to flow into the existing void spaces, resulting in porosity decrease which increases the contact area between the particles. At this point, if the pressure is released, the particles can recover to their former state (elastic deformation and spring-back effect). Continuous application of the pressure leads to particle fracturing into fragments, followed by rearrangement of the fragments (plastic deformation), which may result to mechanical interlocking if the material is brittle.

c) Stages a) and b) progress until the compact density gets close to the specific density of the material. Local melting of the materials occurs if the melting points of the constituents (such as lignin) are reached, causing binding. If this stage is not reached, it can lead to disintegration of the pellets/briquettes.

Densification is an important strategy for the biomass market, because it improves the convenience and accessibility of biomass due to the uniform shape and size. Pelletized LB can be handled more efficiently using the handling and transportation infrastructure of commodity grains, because the handling properties of pellets are similar to grains (Adapa et al. 2009a; Kaliyan and Morey 2009a). If the biomass were to be used for direct combustion purposes, densified biomass pellets provides clean, stable fuel, enhances its volumetric calorific value, and can be easily adopted into the direct-combustion or co-firing with coal using gasification, pyrolysis, and biomass-based conversion reactors (Granada et al. 2002; Mani et al. 2006a; Adapa et al. 2009a; Kaliyan and Morey 2009a). Despite the challenges involve in the handling, storage, and transportation of loose biomass, the direct combustion of loose biomass material in conventional grates is surrounded with very low thermal efficiency. "The conversion efficiencies are as low as 40% with widespread air pollution in the form of very fine particulate" matter (Grover and Mishra 1996). The development of a large-scale biomass unit, necessitate conversion of biomass material into a high-density and high-value solid material like pellets/briquettes (Kaliyan and Morey 2009a; Grover and Mishra 1996). Manufacturing good and quality pellets, briquettes, and cubes is largely thought as an art rather than science by many feed mill operators (Briggs et al. 1999).

Studies have demonstrated that densification technique increases the bulk density and flowability of biomass products, reduces the bulkiness, decreases the porosity, essentially enhances the handling characteristics of the biomass materials for transportation and storage purposes, producing structural useful forms, enhancing appearance, and decreases spillage and wind loss (Briggs et al. 1999; Obernberger and Thek 2004; Adapa et al. 2009b). To reduce the production costs of biofuel, it is of paramount importance to densify the biomass so as to reduce the transportation and storage costs, while increasing the heating value per unit volume.

Densification increases the bulk density of biomass material from an initial bulk density (including baled density) of 40-200 kgm^{-3} to a final bulk density of 600-800 kgm^{-3} (Kaliyan and Morey 2009a). Loose plant-based biomass has low bulk density depending on the plant species, particle density, and particle size. The bulk density of biomass (dried straw) can be as low as 40 kgm^{-3} (Sokhansanj et al. 2005), 40-80 kgm^{-3} for corn stover, and 250 kgm^{-3} for some wood residues (Tabil 2009). Crop straws made into pellets increases in particle density to about 823-1011 kgm^{-3} (Adapa et al. 2009a). Adapa et al. (106b) reported that the mean densities of barley, canola, oat, and wheat straw compacts increased from 907 to 988 kgm^{-3}, 823 to 1003 kgm^{-3}, 849 to 1011 kgm^{-3} and 813 to 924 kgm^{-3}, respectively, with application of pressure between 31.6-138.9 MPa.

Other advantages associated with biomass fuel pellets include: a) reduced amount of emission (dust) and waste produced in consumer end-use; b) reduced storage costs, as they take up less space compared to the non-densified biomass; and c) more efficient combustion control because fuel pellets are uniform in size and their flow into combustion boilers can be better regulated (Grover and Mishra 1996). Cost is a challenge in biomass densification because of the high consumption of energy. Low production costs, high quality pellets and briquettes together with safe handling of biomass will make biomass competitive with fossil fuels (Grover and Mishra 1996).

As earlier mentioned, the structure of lignocellulosic biomass consists of complex molecules of cellulose, hemicellulose, and lignin. Modifying the structural arrangement of cellulose-hemicellulose-lignin matrix during the pretreatment process can improve the binding properties

of lignocellulosic straw biomass, without using any expensive artificial binder. "Polymer becomes more cohesive in the presence of moisture when high molecular amorphous polysaccharides are reduced to low molecular components", (Chen et al. 2004). Most biomass may contain natural adhesives, for example, protein in grasses and forages, lignin in crop straws and wood residues, etc (Sokhansanj et al. 2005; Briggs et al. 1999). When such biomass is subjected to high pressure, these compounds "are squeezed out of the stem and leaf walls, which are responsible for bonding" of compacted particles and stabilization of pellets (Sokhansanj et al. 2005; Briggs et al. 1999). These natural adhesives are released to provide sufficient binding force only within a narrow window of moisture. However, these natural adhesives do not form good pellets with good physical characteristics when pelletized. Sokhansanj et al. (2005) and Briggs et al. (1999) also reported that the hardness and durability of pelletized biomass can be enhanced by optimizing a combination of physico-chemical treatments of biomass during pretreatment and before/during densification processes.

A couple of variable parameters such as the material properties (moisture content, particle size, and chemical constituent), and operating variables (temperature, pressure, and residence time of compression) are responsible for the physical quality (density, durability, and dimensional stability) and variability in energy requirement in the manufacture of biomass pellets and briquettes (Mani et al. 2006a).

2.8.1 Material Properties

2.8.1.1 Effect of Moisture Content

An optimum window of moisture content of biomass is required for the manufacture of dimensionally stable and durable pellets and briquettes. A number of research investigations have been done on this; the results show that the optimum moisture content depends on the type of biomass with respect to the chemical constituents. "Water acts as a binding agent by strengthening the bonding in the material" (Kaliyan and Morey 2009). "Water helps to develop van der Waals forces by increasing the area of contact between particles" (Grover and Mishra 1996). The higher the moisture contents of wafers, the lower the durability (Mani et al. 2006a). At increased moisture content, water acts as a lubricant and decreases the binding characteristics

of the feedstock even at high pressures; this results to low pellet/briquette durability, stability, and density (Mani et al. 2006a). Low moisture chopped corn stover (5-10% w.b.) led to denser, more stable, and durable briquettes as compared to high moisture stover (15% w.b.). A specified amount of moisture develops self-bonding characteristics in LBs at an optimum temperature, pressure, and particle size (Mani et al. 2006a). Colley et al. (2006) also investigated the physical characteristics of the switchgrass pellets. They reported that bulk density, particle density, durability, and hardness of the pellets were extremely affected by moisture content. The optimum moisture content value to obtain good and high values of the above physical characteristics of the pellet was at 8.6% (wet basis).

2.8.1.2 Effect of Chemical Constituents

Livestock feeds with higher amount of starch and protein component generate denser and more stable pellets and briquettes as compared to biomass with higher amount of cellulosic material (Sokhansanj et al. 2005; Tabil (1996) cited that feeds with high natural protein can plasticize under heat generated by friction as the material passes through the die. Materials like dehydrated alfalfa, corn cob and meal, rice bran, and grain screenings with high fiber content produce good quality pellets that do not break easily, because fiber is considered to be a natural binder. However, it presents difficulty to compress and force through the die holes and at the same time, it leads to low production rates (Tabil 1996). Briggs et al. (1999) studied the lubricating effect of oil in the die, and reported that addition of lubricating oil increases the pellet product throughput in a pellet mill. Oil acts as a lubricant between particles and between the biomass feedstock and the die-wall, leading to a lower densification pressure. However, the researchers demonstrated that increasing the oil content above 7.5% greatly decreased two major physical qualities of pellet, durability and hardness (Briggs et al. 1999).

2.8.1.3 Effect of Particle Size

Grinding of biomass into fine particle size is one of the unit operations involved in the densification and pre-processing of biomass as a source of energy (Tabil 1996). It increases the surface area for better pretreatment, densification, and for an efficient biofuel conversion. Finely ground feedstock produces denser pellets and improves the pelleting capacity (Tabil 1996).

Grinders are among the largest power consuming machinery depending on the biomass material, particle size, and the grinding mechanisms (Mani et al. 2004). The design and choice of the grinder are important for reducing the energy input in biomass pre-processing. Usually, the finer the biomass grind, the higher the energy input, the higher is the dimensional stability and durability of the biomass pellets and briquettes (Kaliyan and Morey 2009a). Fine biomass particles usually adsorb more moisture as compared to larger particles; as such, they are involved in a higher degree of conditioning. Large/coarse ground biomass materials tend to produce low durability pellets due to the creation of natural fissure points that may cause cracks and fractures in pellets (Tabil 1996; Kaliyan and Morey 2009a). Mani et al. (2004) investigated the grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass, and concluded that the energy expended for biomass grinding correspondingly increases as the particle size becomes finer. Kaliyan and Morey (2009a) studied the effects of particle size (0.56 to 0.8 mm) on the densification (briquette) properties of corn stover and switchgrass. They reported that decreasing the geometric mean particle size of ground corn stover from 0.80 to 0.66 mm increased the density of briquettes by 5 to 10%. It was also demonstrated that decreasing the particle size of corn stover grind from 0.80 to 0.66 mm increased the durability of briquettes by 50 to 58% at 100 MPa pressure, and by 62 to 75% using 150 MPa at a moisture content of 10%. These researchers stated that decreasing the particle size of corn stover grind from 0.80 to 0.66 mm increased the specific energy consumption from 0.8 to 1.3 MJt^{-1}

2.8.2 Operating Variables

2.8.2.1 Effect of Temperature

Temperature is one of the main operating variables that play a significant role in the stability and durability of the biomass product and energy consumption. During the densification process, heat is being added in two ways; by using the frictional heat from the die or externally by means of preheating of feed materials. Heat is also generated from friction due to compression (Colley et al. 2006). It can be assumed that due to the presence of moisture in the biomass, there is formation of steam under high pressure condition which helps in the hydrolysis of the hemicellulose and lignin fractions of biomass into smaller sugar, lignin products, and other derivatives (Grover and Mishra 1996). When such products are subjected to heat and pressure

during densification process, act as adhesive binders and supply bonding effect (Grover and Mishra 1996). The addition of heat also contributes to the relaxation of the existing fibers in biomass and correspondingly softens its structure, which decreases their resistance. This in turn leads to an increase in the production rate during densification, decrease in wear of the contact parts, and a corresponding decrease in the specific power consumption (Grover and Mishra 1996). However, densification operating temperature should be less than 300^{o}C which is the decomposition temperature of biomass (Grover and Mishra 1996; Ghebre-Sellassie 1989). Under high densification operating pressure and temperature, lignin plays a major role in the bonding of biomass particles. When biomass is subjected to heat (>140ºC), "lignin tends to become soft, melts, and exhibits thermosetting binder resin properties" (Van Dam 2004a). Van Dam et al. (2004b) developed high strength-high density board materials from whole coconut husks, without the addition of chemical binders. The stages involved in the production are steam explosion, extrusion, milling, then followed by hot pressing. The board materials were comparable with to the commercial wood based panels.

2.8.2.2 Effect of Pressure

Pressure also plays an important role in determining the strength and density of pellets and briquettes. The intention of compaction is to increase the closeness between the particles, at the same time increasing the forces and strength between them, so as to provide a more densified material (Grover and Mishra 1996). Densification provides sufficient strength to withstand rough handling. If uniform pressure is not applied throughout the entire volume of the material, it results to variations in density of the pellets/briquettes (Grover and Mishra 1996). Biomass pellet density increases as the applied pressure increases, which is dependent on the moisture content of the biomass. It is important to understand that the physical properties of biomass (which includes density, moisture content, void volume, and thermal properties among others) also influence its binding mechanisms and behavior (Grover and Mishra 1996). "Densification of biomass under high pressure generates mechanical interlocking and increases adhesion among the particles, creating the intermolecular bonds within the contact area" (Tabil 1996; Kaliyan and Morey 2010). The application of external force (pressure) increases the contact area and the strength of the bond among the adhering partners (Colley et al. 2006). Mani et al. (2006a)

reported that applied pressure had the greatest impact on the total energy consumption followed by moisture content. Adapa et al. (2009b) investigated four pressure levels on the densification of barley, canola, oat, and wheat straws. The authors reported that a pressure of 63.2 MPa for barley and wheat, and a pressure of 94.7 MPa for canola and oat produced pellets with the highest density and with the least specific energy consumption. It should be noted that the inclusion of fat/oil (animal or vegetable based) in biomass feedstock resulted to lower pellet durability rating (Briggs et al. 1999). The reason for this is that fat/oil acts as a lubricant among the feedstock particles, and between the feedstock and the pellet mill die-wall. As result of the reduced friction in the die, the pressure in the die corresponding decreased. This resulted to production of biomass pellets with lower durability rating. The hydrophobic characteristics of fat inhibit the binding attributes of the water-soluble compound in the feedstock such as starch, protein, and fiber.

2.9 Conclusion

The limited available sites of enzymatic attack may arise as a result of the tiny average size of the capillaries/pores in LB to permit the entrance of large enzyme molecules, hence, preventing effective enzymatic reaction and confining it to the external surface. As a result, grinding the feedstock to a very small particle size is required before the pretreatment process. Costs and energy consumption for biomass particle size reduction increases geometrically with decreasing biomass particle size. The best option of pretreatment should result in reduction of highly crystalline nature of cellulose and the cross-linking features of lignin, and increasing the surface area for enzymatic reaction. Limiting the formation of inhibitory by-products is important to make the whole process cost effective and economically viable for investors. Different methods, such as physical/mechanical, chemical, physico-chemical, and biological pretreatment can be applied. The positive and negative effects of these various methods have been studied. LB are largely loose and bulky and with difficulty during handling (transportation, storage, and use). Densification is a good process to avert this problem. It is difficult to compare the performance and economics of these various approaches due to differences in the properties of different biomass feedstocks. Hence there is no generic method of pretreatment or pre-processing lignocellulosic biomass into biofuel.

Chapter 3

3. Effect of Alkaline Pretreatment on Chemical Composition of Lignocellulosic Biomass using Radio Frequency Heating

A similar version of this chapter has been published in the peer-reviewed journal Biosystems Engineering and presented at the CSBE/SCGAB 2011 Annual Conference, respectively:

- Iroba, K.L., L.G. Tabil, T. Dumonceaux and O.D. Baik. 2013. Effect of alkaline pretreatment on chemical composition of lignocellulosic biomass using radio frequency heating. *Biosystems Engineering* 116: 385-398.
- Iroba, K.L., L.G. Tabil, T. Canam and T. Dumonceaux. 2011. Radio frequency-alkaline pretreatment of lignocellulosic barley straw. CSBE/SCGAB 2011 Annual Conference, Inn at the Forks, Winnipeg, Manitoba, Paper No. CSBE11-306, July 10-13, 2011.

Contributions of Ph.D. Candidate

The present study resulted in the establishment of baseline approach in the alkaline pretreatment of lignocellulosic barley straw grind. The potential applicability of radio frequency technique as a pretreatment strategy was explored. The biofuel industry can use this novel technology for the disaggregation and disruption of the lignified biomass feedstock prior to the subsequent stage of the process (enzymatic hydrolysis). The breaking down of this lignified bond is necessary to enhance the accessibility and digestibility of the energy potentials (cellulose and hemicellulose). All the experiments, data analysis and writing were performed by Kingsley Iroba with technical assistance of Dr. Tom Canam, while Dr. Lope Tabil, Dr. Tim Dumonceaux and Dr. Oon-Doo Baik provided editorial input. In addition, Dr. Lope Tabil established research collaboration with

Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatchewan where the chemical compositions analysis was performed.

Contribution of this Paper to Overall Study

Knowledge Gap: It is widely accepted that the breaking of the lignocellulosic bonds in biomass will enhance the digestibility and accessibility of the energy potentials (cellulose and hemicellulose). Earlier work has been conducted using different pretreatment methods on biomass. However, perusal of literature shows that little or no work has been done using the radio frequency technique on the pretreatment of lignocellulosic biomass. Previous research also shows that little work has been done on barley straw unlike wheat straw. Radio frequency has inherent advantages of uniform volumetric heat transfer, deep heat penetration within the samples, and can easily scale up. Therefore, the present study was conducted with the objective to explore the applicability of radio frequency heating for the alkaline pretreatment technique in the deconstruction and disruption of lignocellulosic biomass, and to alter its physical and chemical structures. Subsequently, the obtained data will be used to predict the degree of accessibility and digestibility of the energy potentials for the next stage of enzymatic saccharification/hydrolysis.

Justification: There is usually low yield when non-treated/native lignocellulosic biomass (agricultural biomass materials, especially those from straw and stover, and forest residues) is subjected to enzymatic hydrolysis/saccharification due to the high stability of the material to enzymatic or bacterial attacks. Most often, the biodegradation of non-treated lignocellulosic biomass results in low yield of simple sugars (such as glucose, arabinose, galactose, mannose, and xylose) from enzymatic hydrolysis, generally below 20% of the theoretical maximum (Fan et al. 2006b). This is due to the limitations of available sites for enzymatic attack which arises from the fact that the average size of the capillaries in native/non-treated biomass is too small to allow the entrance of large enzyme molecules; as such enzymatic attack is confined to the external surface.

Application of pretreatment such as radio frequency alkaline technique is essential in order to enhance the accessibility and digestibility characteristics of the energy potentials (cellulose and hemicellulose) leading towards production of simple sugars higher than 20% of the theoretical maximum. The accessibility and digestibility characteristics of lignocellulosic biomass can be enhanced by modifying the structure of cellulose-hemicellulose-lignin matrix by application of pre-processing and pre-treatment methods (Sun et al., 2002). It is postulated that the interaction of alkali and lignocellulosic biomass causes saponification of intermolecular ester bonds linkages within the biomass (Sun and Cheng 2002; Feist et al. 1970). This promotes the swelling of lignocellulosic biomass beyond water-swollen dimensions, and favouring increased enzymatic and microbiological penetration into the cell-wall fine structure (Sun and Cheng 2002; Feist et al. 1970). The removal of such linkages increases the porosity of lignocellulosic biomass, leading to an increase in internal surface area (Nlewem and Thrash 2010; Fan et al. 1987), which subsequently helps to separate the structural linkages between lignin and the complex carbohydrates, and disrupts lignin structure (Fan et al. 1987).

3.1 Abstract

The very nature of lignocellulosic biomass presents resistance and recalcitrance to biological and chemical degradation during enzymatic hydrolysis and the subsequent fermentation process. This leads to a very low conversion rate, which makes the process economically unfeasible. In this study, alkaline (NaOH) pretreatment was applied on barley straw, using radio-frequency-based dielectric heating to enhance accessibility and digestibility during the subsequent stage of enzymatic hydrolysis. Three levels of temperature (70, 80, and 90^{o}C), five levels of biomass:NaOH solution ratio (1:4, 1:5, 1:6, 1:7, and 1:8), 1 h soaking time, screen size of 1.6 mm, 1% w/v NaOH concentration, and 20 min residence time were used for the pretreatment. The effect of the alkaline pretreatment was evaluated through chemical composition analysis of the pretreated and non-treated biomass samples. The use of NaOH solution and the biomass:NaOH solution ratio played a vital role in the breakdown of the lignified matrix. The ratio 1:6 at the four temperatures studied was determined to be the optimal treatment conditions. Radio frequency-assisted alkaline pretreatment resulted in lower acid insoluble lignin and higher total acid soluble lignin moieties. Based on the obtained data, we predict that this pretreatment

will decrease the required amount and cost of enzymes by up to 64% compared to using non-treated biomass.

3.2 Nomenclature

AIL acid insoluble lignin

ASL acid soluble lignin

c intercept

C_p specific heat of mass of biomass and NaOH solution (kJ kg^{-1}°C^{-1})

d.b. dry basis (%)

DW distilled water

dT change in temperature

dt change in time

E energy (kWh)

H correction for hydration

ID internal diameter (mm)

m slope

M mass of biomass and NaOH solution (kg)

ns not significant

OD outer diameter (mm)

P power (kW)

t time (h)

T_1 and T_2 the initial and final temperatures (^oC)

TW tap water

W washed

w.b. wet basis (%)

w/v weight per volume

w/w weight per weight

x the required actual concentration of the furfurals or lignins or sugars present in the sample

y the corrected peak area of the furfurals or lignins or sugars

ϕ_{ip} inter-particle porosity

ρ_b bulk density (kg m^{-3})

ρ_p particle density (kg m^{-3}).

ρ_s solid density (kg m^{-3})

ϕ_{wp} porosity within the particles

ϕ_T total porosity

3.3 Introduction

There is a general consensus that global climate change is caused by forced warming resulting from greenhouse gas (GHG) emissions, which is mainly from the combustion of non-renewable fossil fuels (Warren et al. 2007). As part of the strategies to mitigate the above effect, there is a need to reduce the carbon footprint and also enhance sustainability of energy supply. Lignocellulosic biomass is one of the alternatives that is a readily available and renewable source of energy (Demirbas et al. 2009), with annual production of approximately 200 billion tonnes worldwide (Zhang 2008). Lignocellulosic biomass has long been recognised as a potential sustainable source of mixed simple sugars for fermentation to biofuels and production of other biomaterials. Production of bioethanol from cellulosic biomass will help to reduce the augmentation of atmospheric CO_2 concentrations and create a cleaner environment. It will also avoid the competition between food and energy, create job opportunities, and generate revenue for governments both at federal and provincial or local levels.

3.3.1 The Complex Nature of Lignocellulosic Biomass

Lignocellulosic biomass is a composite of cellulose, hemicellulose, and lignin. Lignin (which is normally deposited during crop maturity) is a cell wall cross-linked aromatic polymer. Lignin is composed of phenylpropanoid units binding hemicellulose and cellulose with cellulose positioned at the inner core of the structure (Fan et al. 2006b). Hemicellulose is a carbohydrate polymer composed of 5- and 6-carbon sugars and has a random, branched, and amorphous structure with little strength. Cellulose is a linear polysaccharide of D-glucose residues linked by

β-1,4-glycosidic bonds with a high degree of polymerisation (Ramesh and Singh 1993). The sugars within cellulose and hemicellulose can be accessed for cellulosic bioethanol production by ethanologenic microorganisms (Ramesh and Singh 1993). However, the composite nature of the molecule, particularly the lignin fraction, presents resistance and recalcitrance to biological and chemical degradation during enzymatic hydrolysis/saccharification and the subsequent fermentation process. This leads to a very low conversion rate, which makes the process economically unfeasible (Söderström et al. 2003; Fan et al. 2006b; Chandra et al. 2007; Taherzadeh and Karimi 2008). Sokhansanj et al. (2006) studied the production and distribution of cereal straw (wheat, barley, oats, and flax) in the Canadian prairies over a period of 10 years (1994-2003). They reported that the provinces of Alberta, Saskatchewan, and Manitoba collectively produced roughly 37 million tonnes of wheat, barley, oat, and flax straw annually. Lignocellulosic agricultural biomass residues represent an abundant, inexpensive and readily available source of renewable lignocellulosic biomass (Lynd et al. 1999; Liu et al. 2005). Canada as a nation is vast in agriculture with barley production as the second largest crop behind wheat (Food and Agriculture Organization, 2007). This implies that Canada will play a very significant role in the development of the biorefinery sector because of its contribution as a predominant supplier or source of biomass feedstock. Barley straw has a higher proportion of cellulose (40%) than wheat straw (30%), although wheat straw has a higher proportion of hemicellulose (50%) than barley straw (20%), with both substrates having similar proportions of lignin (15%) (Ramesh and Singh 1993; Jørgensen et al. 2007). Perusal of the literature shows that a lot of research has been done on wheat straw; therefore, there is a need to explore the potential of barley straw for the biofuel industries. Hence, for these reasons as well as its higher cellulose content than wheat straw, barley straw was chosen for this work.

To overcome the aforementioned challenges, pretreatment strategies are employed to deconstruct/disrupt the lignocellulosic matrix, and possibly decrease the crystalline nature of cellulose, so that the cellulose and hemicellulose can be more efficiently hydrolysed by enzymes (Taherzadeh et al. 2008). A series of stages is involved in cellulosic ethanol production: 1) particle reduction via grinding to increase the surface area for reactions (Fan et al., 2006b); 2) pretreatment using different methods to break down the lignified structure to increase the

enzymatic digestibility of cellulose and hemicellulose (Taherzadeh et al. 2008); 3) hydrolysis/saccharification which converts the complex carbohydrates (cellulose and hemicellulose) into simple sugars such as pentoses (xylose and arabinose) and hexoses (glucose, galactose, mannose) (van Wyk 1997; Kumar et al. 2009); and finally, 4) fermentation of the simple sugars to produce cellulosic bioethanol which is further separated via distillation (Chung et al. 2005). Several technologies have been developed in the past years for the conversion process and pretreatment to enhance the release of the energy potential present in lignocellulosic biomass. Some of the pretreatment methods include: steam explosion, biological, wet oxidation, acid hydrolysis, microwave-chemical, supercritical CO_2 explosion (SC- CO_2), and ammonia fibre explosion (AFEX) pretreatment (Montane et al. 1998; Okano et al. 2005; Palonen et al. 2004; Saha et al. 2005; Zhu et al. 2006; Kim and Hong 2001; Alizadeh et al. 2005). The above studies have shown that there are different shortcomings associated with each of these pretreatment methods. Recently, the clear goal is to make pretreatment of biomass cost-competitive, economically viable, free from production of inhibitors for microbial ethanol fermentation, and easy to scale up.

3.3.2 Radio Frequency Heating Technique

Radio frequency (RF) heating has been considered as an energy efficient heating method that is an alternative to conventional methods (such as hot air and infrared heating). In RF heating, there is a volumetric heat generation inside the product, which is a result of the interaction between the RF waves and the ions/molecules of the product (Piyasena et al. 2003). Therefore, heat flows from inside the product to the outside, unlike conventional heating methods in which heat is transferred from the heating medium to the product via conduction or convection (Donepudi and Muthukumarappan 2009). Microwave (MW) heating has the same principle and mechanisms of heating as RF heating. Microwave (MW) radiation has been applied on the pretreatment of lignocellulosic biomass (Zhu et al. 2006; Zhu et al. 2005; Donepudi and Muthukumarappan 2009). However, RF heating has the following advantages over the MW heating: 1) uniform electric field strength inside the application chambers, therefore preventing uncontrolled heating, overheating, local hot spots, and product degradation; 2) large penetration depth (10-30 m); and 3) higher energy efficiency (Izadifar et al. 2009; Piyasena et al. 2003; Balakrishnan et al 2004).

In an RF chamber, the product is placed between two parallel electrodes (plates) which generate a uniform electric field around the product. In RF and MW heating, heating is based on the product's ability to absorb electromagnetic radiation and convert it to heat (Ryynanen 1995). Applications of MW and RF improve product quality, shorten the processing times, and reduce floor space when compared to conventional heating methods (Ramaswamy and Tang 2008). RF heating is influenced by a number of system specific factors such as frequency, temperature, and product properties such as viscosity, water content, and chemical composition. These affect the dielectric properties and subsequently the RF heating of product (Ryynanen 1995; Piyasena et al. 2003). RF has been successfully applied to leather drying (Balakrishnan et al. 2004), thermal therapy (Brausi et al. 2004), and in other research fields such as food processing (blanching, tempering, pasteurisation, sterilisation) and medicine (Piyasena et al. 2003). Recently, Izadifar et al. (2009) demonstrated that RF can be used for the extraction of podophyllotoxin from rhizomes of *Podophyllum peltatum*. RF heating has been used as a treatment method to inhibit the growth of fungus in wood samples in an industrial 40 kW dielectric oven at temperatures between 60°C and 70°C for 2 min. The results show that RF radiation can inhibit fungus by 98-100% (Tubajika et al. 2007). Piyasena et al. (2003) reported that RF dielectric heating is advantageous over conventional heating for large size food particles. The same authors cited that RF heating has advantages over conventional drying and microwave drying of cookies, crackers and snack foods. Hu and Wen (2008) reported that microwave-assisted alkali pretreatment total sugar (xylose + glucose) yield from the combined treatment and hydrolysis (using cellulase enzymes) was 34.5 g/100 g biomass, equivalent to 58.5% of the maximum potential sugars released. This yield was 53% higher than that obtained from conventional heating of switchgrass.

Perusal of literature showed that Hu et al. (2008) investigated the pretreatment of switchgrass using radio-frequency (RF)-based dielectric heating in alkaline (NaOH) solution to enhance its enzymatic digestibility. These authors used the solid content of switchgrass as a parameter to improve the RF pretreatment performance. Hu et al. (2008) reported that RF heating can pretreat switchgrass at higher solid contents (20-50%) which may not be suitable for the conventional heating method due to poor mixing of the samples and loss of fluidity. The RF band has a frequency range of 3 kHz to 300 MHz, but typically 13.56-27.12 MHz and 40.68 MHz are used

(Ryynanen 1995; Piyasena et al. 2003). The above mentioned advantages associated with RF can enhance its commercialisation and industrial application as a biomass pretreatment method as it can easily be scaled up.

Pre-hydrolysis treatment of lignocellulosic biomass straw, using RF with low concentration of NaOH solution as catalyst, can breakdown the cementing lignin matrix, and subsequently enhance the accessibility and digestibility of the energy potentials (cellulose and hemicellulose), and improve the enzymatic hydrolysis rates of lignocellulosic biomass for biofuel production. Therefore, the main objective of this research was to explore the applicability of RF heating for the alkaline pretreatment technique for the deconstruction and disruption of lignocellulosic biomass barley straw grind, and to alter its physical and chemical structures. Subsequently, the obtained data will be used to predict the degree of accessibility and digestibility of the energy potentials for the next stage of enzymatic saccharification/hydrolysis.

3.4 Material and Methods

3.4.1 Sample Collection and Preparation

Barley straw was obtained in October, 2009 from RAW Ag Ventures Limited, Maymont, SK. The straw was ground using a hammer mill (Serial no. GM13688; Glen Mills Inc., Maywood, NJ) with screen sizes of 0.8 and 1.6 mm to increase the surface area of the biomass. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation, and to provide flow of the biomass in and out of the hammer mill. The initial moisture content of the straw was 8.8% (d.b.). The moisture content was measured based on ASABE standard method, ASAE S358.2 DEC1988 (2008).

3.4.2 Particle, Bulk, and Solid Density Measurement

The particle density of the samples was measured using the gas multi-pycnometer (model No. MVP-2; QuantaChrome, Boynton Beach, FL) by calculating the displaced volume of nitrogen gas by a known mass of material. Three replicate tests were performed on each sample. The bulk density of the samples was determined by passing the material through a funnel which was placed above a standard 0.5 l steel cup (SWA 951, Superior Scale Co. Ltd., Winnipeg, MB).

Blockages in the funnel during ground biomass flow were cleared using a thin steel rod. After filling the cup, the excess sample was removed by passing a steel roller in a zigzag manner over the top of the cup. The mass contained in the cup was then determined. Bulk density was calculated by dividing the mass of material within the cup by the volume of the cup. Three replicates were performed for each sample.

The inter-particle porosity of the ground samples was computed from the particle and bulk densities using equation 3.1. To better understand the physical attributes of the barley straw grind, the screen size was further decreased to 0.354 mm. Therefore, the particle and bulk density were determined for samples of barley straw grind using hammer mill screen sizes of 0.354, 0.8, and 1.6 mm.

$$\phi_{ip} = 1 - \frac{\rho_b}{\rho_p} \tag{3.1}$$

where ϕ_{ip} is the Inter-particle porosity, ρ_b is bulk density (kg m^{-3}), and ρ_p is particle density (kg m^{-3}).

The particle density or true density of a particulate powder or solid, is the density of the particles that make up the powder or solid, whereas the solid density is the density of the particles that make up the powder or solid with minimal or no voids/porosity within the particles. In order to further investigate these physical properties of the biomass samples, the solid density of barley straw grind was determined. Using helium in the gas multi-pycnometer, the solid density of barley straw finely ground using a hammer mill fitted with a 0.354 mm opening screen was determined. Helium gas is a low molecular weight gas that can penetrate and diffuse through solids quickly and easily. Helium gas gives detailed information on the void fraction/porosity of the bed, as well as the voids within the single particles. The data obtained were used to calculate the solid density. While the obtained data using nitrogen gas with the original biomass grinds were used to calculate the particle density as mentioned above. Three replicates were also performed for the solid density measurement. The porosity within the particles was calculated using equation 3.2:

$$\phi_{wp} = \frac{\rho_s - \rho_p}{\rho_p} \quad (3.2)$$

where ϕ_{wp} is the porosity within the particles and ρ_s is the solid density (kg m^{-3}).

Total porosity, ϕ_T was calculated by summation of the inter-particle porosity and porosity within the particles.

3.4.3 Radio Frequency Pretreatment

Preliminary experiments revealed that NaOH concentration, biomass screen size, soaking time, and the ratio of biomass to NaOH solution have significant effects on the dielectric properties of RF biomass pretreatment process (see appendix A). Hence, the optimum contributing variables were selected for RF biomass pretreatment. The pretreatment was performed using an RF machine (1.5 kW and 27.12 MHz laboratory dryer, Strayfield, Theale, Reading). The pretreatment was done in two phases. The first phase (using a Lexan polycarbonate reactor, see Appendix C) was used to optimise the process variables in Table 3.1a. Based on the obtained results from the optimisation phase (see Appendix C), the optimal hammer mill screen size and NaOH solution concentration were identified. To understand further the interactions between the process and material variables, the second phase was based on a factorial experiment in a completely randomised design. The variables shown in Table 3.1b and a blown glass reactor (volume 4.25 l) with the dimensions shown in Figure 3.1 were used. Higher temperatures (70^{o}C, 80^{o}C, and 90^{o}C) were used in phase two.

Table 3.1a Material and operating variables using Lexan polycarbonate reactor

Variables	Levels
Screen size	0.8 and 1.6 mm
NaOH solution	0.5 and 1% w/v
Biomass: NaOH solution	1:6 - 35 g biomass and 210 g NaOH
Soaking time	1 hour
Temperature	40, 50, and 60 ^{o}C
Residence time	20 minutes

Table 3.1b Material and operating variables using blown glass reactor

Variables	Levels
Hammer screen size	1.6 mm
NaOH solution concentration	1% w/v
Biomass: NaOH solution ratio	1:4 - 110 g biomass and 440 g NaOH solution 1:5 - 110 g biomass and 550 g NaOH solution 1:6 - 100 g biomass and 600 g NaOH solution 1:7 - 100 g biomass and 700 g NaOH solution 1:8 - 90 g biomass and 720 g NaOH solution
Soaking time	1 h
Temperature	70, 80, and 90 ^{o}C
Residence time	20 minutes

The focus of this experimental design was to investigate the effects of the independent variables as listed in Table 3.1b on the following dependent variables: cellulose (glucose), hemicellulose (xylose, arabinose, mannose, and galactose), lignin (soluble and insoluble lignin moieties), the furfurals (5-hydroxyl-methyl furfural and furfural), and ash content. The basic understanding of the changes in these chemical compositions will assist in evaluating the efficiency of the RF-alkaline pretreatment process.

A blown glass reactor was used in phase two because of the critical challenges that were encountered during the optimisation stage with the Lexan polycarbonate reactor. There was arcing and creation of hot spots due to the interaction between the Lexan polycarbonate composition, NaOH solution, and the generated electromagnetic field. This led to a non-uniform heat distribution profile (results/graphics not shown).

The biomass samples and the NaOH solution were mixed thoroughly in the reactor based on the ratio indicated in Table 3.1b. Selective heating (due to presence of aqueous solution which results in excitation to different levels at various locations within the reactor) can also be minimised by adequate mixing. The surface of the bulk of the mixture in the reactor was carefully smoothed to avoid spikes, which could cause arcing, sparks, and local hot spots. One hour soaking time at room temperature was allowed before subjecting the mixture to RF heating. The reactor containing the mixture was then placed between the RF male (top) and female (bottom) electrodes with 191 mm clearance.

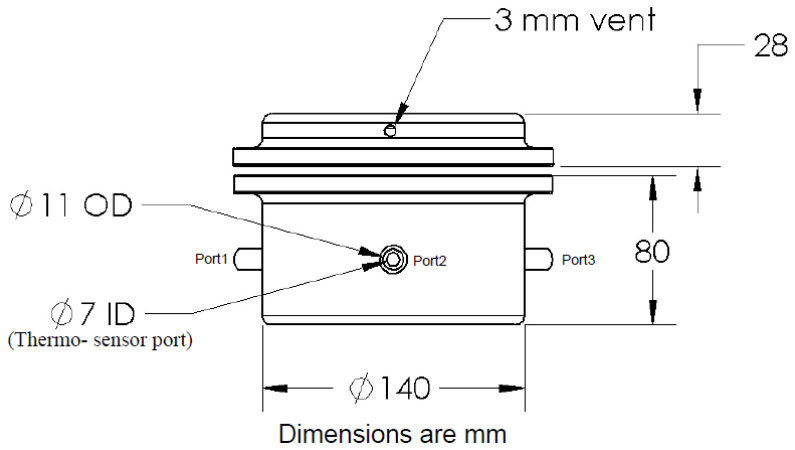


Figure 3.1 Schematic diagram of blown glass reactor: ID – internal diameter, OD – outer diameter. The vent is use for pressure release, to avoid pressure build up within the reactor.

The distance between the male electrode and surface of the biomass-NaOH solution mixture was 115 mm. Three equally spaced electrode bars were attached to the male electrode (Figure 3.1), the distance between the bars and the surface of the biomass-NaOH solution mixture was 42 mm. A 20 min retention time at the pre-set temperature was used. Each treatment combination was duplicated. Three fibre optic temperature sensors were connected to the reactor at three different locations/ports as shown in Figures 3.1 and 3.2 to monitor the radial heat distribution within the reactor. Figure 3.2 shows the blown glass reactor with the sample inside the RF machine. The temperature sensors were positioned 30 mm from the wall of the reactor in the bulk of the sample at 120^{o} from each other. A power meter was connected to the RF set up to monitor the power consumption.

3.4.4 Data Acquisition and Control

Data acquisition and logging in this experiment were done using LabView (National Instruments, Austin, TX). The data logging was one data point for every 2 s. Temperature, power, and time were converted to degree Celsius (^{o}C), kW, and seconds, respectively.

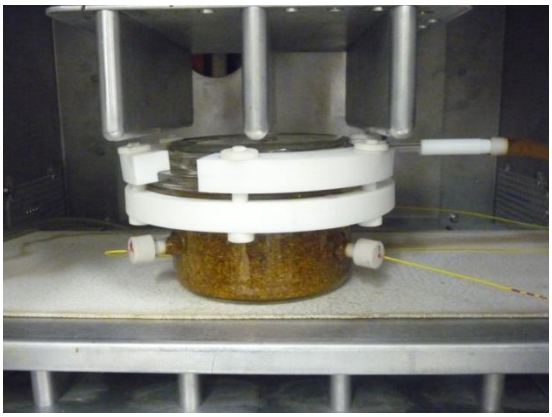


Figure 3.2 Blown glass reactor containing the biomass-NaOH solution mixture inserted between two electrodes of the RF machine.

3.4.5 Washing of the RF Alkaline Pretreated Samples

Four treatment combinations were randomly selected for washing to get rid of the NaOH solution in the RF-assisted alkaline pretreated samples. After heating the mixture of biomass-NaOH solution in the RF machine, it was placed in a vacuum filter consisting of a perforated porcelain funnel 200 ml with Whatman filter paper (size 40). Tap water (approximately 1 l) was repeatedly used for the washing until the pH reached around 7.0. The RF-assisted alkaline pretreated washed samples were dried to 10% moisture (w.b.) using the forced-air convection dryer (Shaw et al. 2007) set at 40^{o}C. To evaluate the efficiency of the RF alkaline pretreatment process, chemical composition analysis of the pretreated and non-treated samples was carried out.

3.4.6 Chemical Composition Analysis

The National Renewable Energy Laboratory standard (NREL) (Sluiter et al. 2007) was used for the chemical composition analysis. The NREL standard uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The first step uses 72% H_2SO_4, while the second step uses 4% H_2SO_4. Prior to this analysis, the biomass sample at 10% (w.b.) was dried at 105^{o}C in an air-oven (Shel-Lab model No. 1350F; Sheldon Manufacturing Inc., Cornelius, OR) for 24 h. The non-structural carbohydrates/extractives (nitrites, protein, ash, waxes, chlorophyll, etc.) were extracted using a Soxhlet apparatus and refluxed with acetone

under heat for 24 h prior to the chemical composition analysis. The extractive-free biomass was then left at room temperature for about 3-4 h so that the acetone could evaporate; the samples were dried at 105^{o}C in an air-oven for 24 h. Approximately 300 ± 10 mg of each dried biomass was used for the chemical analysis. Chemical composition analysis was done on the structural carbohydrates and lignin of the RF alkaline pretreated, washed, and non-treated biomass. The lignin fractionates into acid insoluble and acid soluble material. The acid insoluble lignin (AIL) is the residue (remaining solids) from the hydrolysis suspension. The crucible and the residue were dried at 105^{o}C for 24 h to a constant weight in a drying oven and subsequently cooled in a desiccator before the weight was recorded. Thereafter, AIL was obtained based on the NREL protocol. The acid soluble lignin (ASL) was measured using two different NREL standard approaches; the first was using UV-Vis spectroscopy (Helios Aquamate v7.09 spectrophotometer, Thermo-Scientific, Cambridge, England).

The second approach for measuring the ASL was done using the Ultra Performance Liquid Chromatography (UPLC), which has the capability of separating and quantifying the various lignin components. An aliquot of approximately 30 ml (filtrate) of hydrolysate was neutralised by adding 1 g of $CaCO_3$, mixed, allowed to settle, and subsequently centrifuged for 2 min at 1500 rev min^{-1}. This was set aside and stored at -20^{o}C before monosaccharide and lignin quantifications using the Waters Acquity UPLC–MS system (Acquity 2004-2010, Waters Corp., Milford, MA). The LC conditions for the monosaccharide quantification were: Acquity UPLC BEH Amide column (1.7 µm pore size, 2.1 x 50 mm); 0.25 ml min^{-1} flowrate; mobile phase A: 95% acetonitrile/5% isopropanol; mobile B: 80% acetonitrile/0.1% NH_4OH; gradient from 100% A to 100% B over 10 min, then gradient of 100% B to 100% A over 4 min (14 min total run time/sample). The MS conditions for the monosaccharide quantification were: 2.8 kV; 25 V (cone); 50 l h^{-1} (cone); gas flow 600 l h^{-1}; desolvation temperature 350^{o}C; source temperature 120^{o}C; and dwell time of 0.08 s. The LC conditions for the acid-soluble lignin moieties quantification were: Acquity UPLC BEH C18 column (1.7 µm pore size, 2.1 x 50 mm); 0.25 ml min^{-1} flowrate; mobile phase A: water/0.1% formic acid; mobile phase B: acetonitrile gradient from 95% A to 65% A over 4 min, then gradient of 5% A to 95% A over 4 min (8 min total run time/sample).

The stored sample was further prepared for the UPLC monosaccharide quantification analysis by adding 800 µl of 75% acetonitrile/25% methanol and 100 µl of fucose solution (~1 mg/ml into 100 µl of the neutralised hydrolysate, filtered through 0.2 µm filter into 2 ml UPLC vial. For the lignin quantification analysis, the stored sample was prepared by adding 800 µl 50% water/50% methanol and 100 µl O-anisic acids (1 mg/ml) into 100 µl of the neutralised hydrolysate, filtered through 0.2 µm filter into 2 ml UPLC vial.

During the two-step acid hydrolysis, the polymeric carbohydrates (cellulose and hemicelluloses) were hydrolysed into monomeric forms (xylose, arabinose, mannose, glucose, and galactose), which are soluble in the hydrolysis liquid. These were measured by UPLC. The standards of the above monomeric sugars were prepared and also analysed using the UPLC for regression fitting purposes. The spectra of xylose and arabinose displayed at a molecular weight of 149.1 g $mole^{-1}$ while the mannose, glucose, and galactose displayed at a 179.2 g $mole^{-1}$. The fucose was added and collected at a molecular weight of 163.2 g $mole^{-1}$. Fucose is a standard used for normalisation during the regression stage of the analysis.

The furfurals: 1) 5-hydroxyl-methyl furfural; and 2) furfural, and the lignin moieties (components): 1) H acid lignin group (4-hydroxybenzoinc acid); 2) G acid lignin group (vanillic acid); 3) S acid lignin group (syringic acid); 4) H aldehyde lignin group (4-hydroxybenzaldehyde); 5) G aldehyde lignin group (vanillin); and 6) S aldehyde lignin group (syringaldehyde) were also measured using the UPLC. The peaks of the first 5 components above were analysed at a wavelength of 265 nm and the last 3 components at a wavelength of 285 nm. The standards of the above lignin components were also prepared and analysed using the UPLC for regression fitting purposes. The O-anisic acid was added as a standard used for normalisation during the regression stage of the analysis and the peak was collected at a wavelength of 285 nm. The spectra generated from the UPLC were integrated and extracted using Empower 2 software (Waters Corp., Milford, MA). The extracted integrated peak area was correlated to the concentration of the standards using a pre-determined regression equation from dilution series using Microsoft Excel. The actual concentration of the furfurals and lignins present in the samples were computed by using $y = mx + c$; where m = slope, c = intercept, and x

= the required actual concentration of the furfurals or lignins present in the various samples, and y = the corrected peak area of the furfurals or lignins, therefore, x = (y - c)/m. The obtained values were corrected for dilution factors (10 and 87 ml) and divided by the original initial mass of the oven-dried samples (300 ± 10 mg) to ascertain how much of the various compounds was present in the initial dried samples as shown in equation 3.3

$$\text{Lignin content} = \frac{\text{lignin moieties concentration x 87 mL x 10}}{\text{dried sample mass}} \quad (3.3)$$

where 10 is the dilution factor of 1/10 that was used during the preparation of the samples for UPLC analysis and 87 ml is the actual volume of the autoclaved sample.

For the monomeric sugars regression analysis, the same regression approach was followed, however, fucose was used in place of O-anisic acid for the normalisation process. The sugars were computed using equation 3.4:

$$\text{Sugar content} = \frac{\text{sugar concentration x 87mL x 10 x H}}{\text{dried sample mass}} \text{x } 100\% \quad (3.4)$$

H could be 0.88 or 0.90 depending on the number of carbons present in the sugars. H accounts for the water molecule that was added during the hydrolysis. The 5-carbon sugar values (pentoses: xylose and arabinose) and the 6-carbon sugars (hexoses: mannose, glucose, and galactose) were multiplied by anhydro correction factor of 0.88 and 0.90, respectively. Each sample was replicated twice.

3.4.7 Determination of Ash in Biomass

The ash content, which is a measure of the mineral content and other inorganic matter (structural or extractable) in biomass, was measured as part of the total composition. Structural ash, which is inorganic material, is bound in the physical structure of the biomass, while the extractable ash is the inorganic material that can be removed by washing or extracting the material (Sluiter et al. 2008). The ash content of the biomass samples were determined based on National Renewable Energy Laboratory standard (NREL) (Sluiter et al. 2008). About 0.5-2.0 g of the oven dried samples (dried @ 105^{o}C overnight) was weighed into the tared dried crucible. The weighed

crucible and the sample were placed in a muffle furnace (Model 650-14, Fisher Scientific, *Pittsburgh,* PA, U.S.A.) equipped with a thermostat with ramping program. Samples were heated to 105^{o}C, held at 105^{o}C for 12 min, ramped to 250^{o}C at 10^{o}C min^{-1}, held at 250^{o}C for 30 min, ramped to 575^{o}C at 20^{o}C min^{-1}, held at 575^{o}C for 180 min. Then the temperature was allowed to drop to 105^{o}C and held at 105^{o}C until the samples were removed. The samples were carefully removed from the furnace and directly placed into a desiccator and cooled. The crucible and the ash were weighed. The ash content was computed as the percentage of residue remaining after the dry oxidation at 575^{o}C using equation 2.5

$$\%\text{Ash} = \frac{\text{weight}_{\text{crucible plus ash}} - \text{weight}_{\text{crucible}}}{\text{oven dried weight}_{\text{sample}}} \text{x} 100\% \quad (3.5)$$

3.4.8 Statistical Analysis

Experimental data were statistically analysed via analysis of variance (ANOVA) using the general linear model (univariate). It was conducted at 5% significance level using SPSS (Superior Performing Statistical Software version 14 for Windows, 2005; IBM, Armonk, New York, NY).

3.5 Results and Discussion

3.5.1 Physical Characteristics of Lignocellulosic Biomass

Table 3.2 depicts the physical characteristics of the non-treated biomass grind. As the hammer mill screen size decreased, the particle and bulk densities increased. There might be some effect of moisture transfer and compaction during the grinding process that may be responsible for the differences in the particle density for the different hammer mill screen sizes. Adapa et al. (2011a) investigated the grinding performance and physical properties of non-treated and steam exploded barley, canola, oat, and wheat straw. They reported that the particle density significantly increased with a decrease in hammer mill screen size from 30 to 1.6 mm. Mani et al. (2004) performed a similar investigation on the grinding performance and physical properties of wheat

and barley straws, corn stover, and switchgrass and reported a similar trend of particle density with respect to hammer mill screen size.

Table 3.2 Physical attributes of barley straw grind

Physical characteristics	Hammer mill screen size		
	0.354 mm	0.8 mm	1.6 mm
Particle density (kg m^{-3})	1323.22 (1.94)	1272.72 (0.59)	1126.57 (2.14)
Bulk density (kg m^{-3})	130.36 (0.89)	124.75 (0.19)	98.35 (0.76)
Inter-particle porosity (%)	90 (0.00)	90 (0.00)	91 (0.00)
Solid density (kg m^{-3})	1358.19 (5.26)	-	-
Porosity within particle (%)	30 (0.00)	-	-
Total porosity (%)	120	-	-

Values in parentheses = standard error; number of replicates, n = 3

The use of helium gas in the gas pycnometer produced higher values of density (solid density) because of its capability to penetrate into the voids between the particles and the voids within the particles, unlike the higher molecular weight nitrogen gas, which can only penetrate the void fraction between the particles to measure the particle density. In a nutshell, particle density only considers the voids/porosity between particles, while solid density put into consideration voids/ porosity between particles and within particles. The inter-particle porosity was about three times higher than the porosity within the particles. This implies that the voids within the particles were much smaller than the void spaces between the particles, which are dependent on the particle size.

3.5.2 Radio Frequency Pretreatment

The addition of NaOH to lignocellulosic biomass causes swelling of particles within the mixture. It is postulated that the mechanism is the interaction of alkali and lignocellulosic biomass causing saponification of intermolecular ester bonds linkages within the biomass (Sun and Cheng 2002; Feist et al. 1970). This promotes the swelling of lignocellulosic biomass beyond water-swollen dimensions, and favouring increased enzymatic and microbiological penetration into the cell-wall fine structure (Sun and Cheng 2002; Feist et al. 1970). The removal of such linkages increases the porosity of lignocellulosic biomass, leading to an increase in internal

surface area (Nlewem and Thrash 2010; Fan et al. 1987), which subsequently helps to separate the structural linkages between lignin and the complex carbohydrates, and disrupts lignin structure (Fan et al. 1987). Figure 2.3 shows that there was uniform heat distribution during RF alkaline pretreatment with no thermal runaway or local hot spot detected within the reactor. The temperature recorded in ports 1-3 reached the preset temperature (80^{o}C) at approximately the same time (104 min).

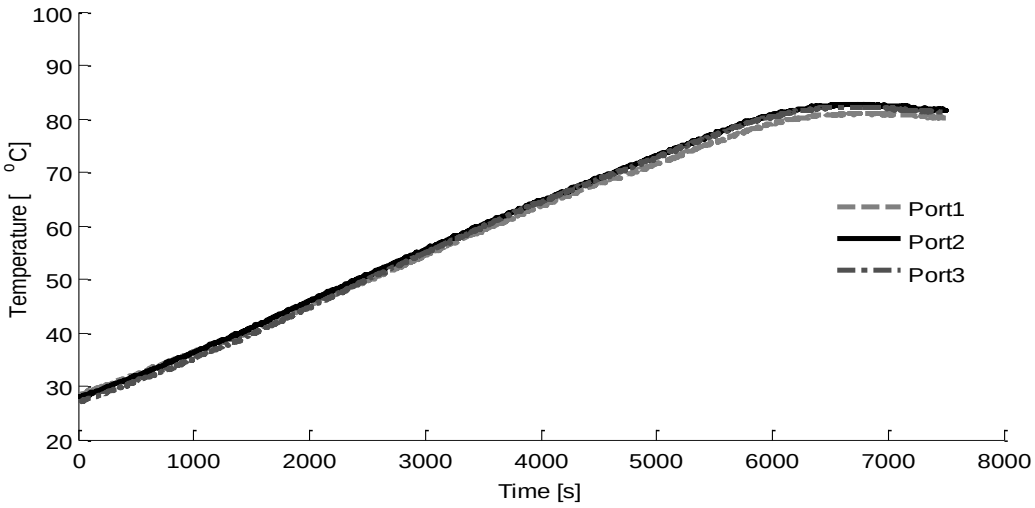


Figure 3.3 Temperature profile of biomass-NaOH solution (1% w/v) at 80^{o}C, 1.6 mm hammer screen size, and biomass: NaOH solution ratio of 1:8 using the blown glass reactor. Ports 1-3 represent the radial temperature profile taken from the three ports of the reactor as shown in Figures 3.1 and 3.2.

Table 3.3 shows that as the ratio of biomass to NaOH solution decreased from 1:4 to 1:8, there was a corresponding increase in the heating time and the energy consumption, with a decrease in the heating rate. This is due to the higher volume of the NaOH solution in the mixture, which leads to differences in the specific heat. The higher biomass:NaOH solution ratio required less energy to heat the mixture. This implies that increasing the volume of the NaOH solution does not contribute to the increase in the concentration of the NaOH solution for the RF heating, but only increases the mass density of the NaOH solution; as such it requires more energy and time to heat up the increased mass of the system. RF is not the best for liquid heating (and lower ratio mixtures approach liquid heating). Selective heating (which is due to presence of aqueous

solution that could results in excitation to different levels at various locations within the reactor) can be minimised by adequate mixing. Therefore, due to the unique characteristics of RF heating (i.e., volumetric heat generation/transfer, deep heat penetration and energy efficiency), biomass could be treated on a large scale using RF, at high solids content, and with a uniform temperature profile, unlike the conventional heating that requires high liquid content. RF heating is accompanied by heat loss to the surrounding space through natural convection and radiation which reduces the temperature gradient significantly. Thus, RF heating of biomass is more efficient for higher biomass:NaOH solution ratios. The long heating time observed in this investigation is also a result of the low power level (1.5 kW) of the laboratory RF machine and the protruding space on the lid of the blown glass reactor used, which created a gap/space between the top level of the prepared biomass-NaOH solution mixture and the lid of the reactor. Space decreases dramatically the electric field strength generated between the RF electrodes as air acts as resistance. As such, it took more time to heat the mixture. Therefore, for future and scale-up purposes, to increase the heating rate, RF at higher power levels should be used and the reactor needs to be redesigned so as to avoid any space/gap between the surface of the biomass-NaOH solution mixture and the lid of the reactor. The distance between the surface of the biomass-NaOH solution mixture and the male (top) electrode should be at a minimum. The available RF power level for commercialisation is now up to several hundred kW. The reported energy consumption in Table 3.3 includes the heating period to the preset temperature and the residence time at which the pretreatment temperature was maintained. The energy (E) in kWh is equal to the area under the power (P, kW) versus time (t, h) curve. The theoretical energy consumption (Q) was also calculated based on lumped heat equation at the five levels of ratio of biomass:NaOH solution:

$$Q = MC_p(T_2-T_1) \quad 3.6$$

where M = mass of biomass and NaOH solution (kg) (see Table 3.3); C_p specific heat of mass of biomass and NaOH solution (kJ $kg^{-1}°C^{-1}$) (Iroba et al. 2013c) and T_1 and T_2 are the initial (24^oC) and final (90^oC) temperatures of the biomass and NaOH solution mixture.

The results obtained showed that the calculated theoretical energy required for the pretreatment based on equation 3.6 above is much lower than the actual energy measured. The estimated theoretical energy requirement for heating are 0.030, 0.040, 0.043, 0.045 and 0.045 kWh for ratios of biomass to NaOH solution of 1:4, 1:5, 1:6, 1:7 and 1:8, respectively.. There is a large difference between the actual and theoretical energy consumption. The actual energy consumed is 29 times higher than the theoretical energy consumed for ratio 1:4, and 31, 44, 48 and 50 times higher than the theoretical energy for ratios 1:5, 1:6, 1:7 and 1:8, respectively.

Table 3.3 Heating rate and energy consumption during the RF alkaline pretreatment using the blown glass reactor

Temperature (^oC)	Biomass: NaOH solution ratio	Heating time to the preset temperature (min)	Mass of biomass:NaOH solution mixture (g)	dT/dt to the preset temperature ($^oC\ s^{-1}$)	Energy consumed (kWh)	Estimated energy consumed per tonne of biomass:NaOH solution mixture ($kWh\ t^{-1}$)
90	1:4	32	550	0.0449	0.88	1604
90	1:5	48	660	0.0448	1.24	1880
90	1:6	80	700	0.0280	1.90	2710
90	1:7	99	800	0.0332	2.14	2680
90	1:8	105	810	0.0268	2.23	2757

dT change in temperature and dt = change in time; The heating rate (dT/dt) was calculated for the time taken to reach the preset temperature, excluding the hold (residence) time.

This large difference in values between the actual energy required and the calculated theoretical energy consumption confirms that the RF machine needs to be optimized so as to increase the heating rate and decrease the energy consumption.

3.5.3 Chemical Composition Analysis of RF-Alkaline Pretreated and Non-Treated Barley Straw Grind

This investigation shows that lignocellulosic biomass absorbs more NaOH than water, because of the hydrophobic nature of lignin, which acts as an external crosslink binder on the biomass matrix and shields the hydrophilic structural carbohydrates (cellulose and hemicellulose). The results obtained from the first phase (optimisation stage: see Appendix C for results) of the

experiment showed that there was no significant difference between the acid soluble lignin moieties and insoluble lignin obtained from biomass ground using hammer mill at 0.8 and 1.6 mm screen size RF alkaline pretreated biomass; as such, 1.6 mm screen size was adopted for this current investigation. This minimises energy use in particle size reduction. The study also demonstrated that NaOH concentration is a major factor in the pretreatment process. Biomass samples pretreated with 1% w/v NaOH produced higher acid soluble lignin moieties with corresponding lower acid insoluble lignin when compared to 0.5% w/v NaOH pretreated samples. Statistical analysis shows that concentration of NaOH solution has a significant effect ($P<0.05$) on the acid soluble lignin. Hence, 1% w/v NaOH was used for this investigation. The swelling initiated by NaOH creates pores in the biomass matrix (Nlewem and Thrash 2010; Fan et al. 1987), which helps enhance the reactivity of the biomass matrix to any externally added material such as enzyme. A similar finding was reported by Kumar et al. (2009). Table 3.4a shows that there is a reduction in the acid insoluble lignin in the RF alkaline pretreated samples as compared to the non-treated samples. Lignin removal is an important part of the pretreatment process, because lignin can effectively inhibit/prevent the cellulase enzymes from hydrolysing the cellulose. This lignin reduction resulting from the alkaline pretreatment has also been reported by Xu et al. (2009) and Silverstein et al. (2007). The structural separation, disruption and disaggregation initiated by the interaction between the biomass and NaOH solution in the presence of the RF heating is believed to be responsible for this solid loss. The disruption and deconstruction of the lignified matrix is also associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF and the ions and molecules from the NaOH solution and the biomass. During the pretreatment process, the ester bonds between lignin and the complex carbohydrates are disrupted (Sun et al. 2002). Some portion of lignin is broken down, degraded, and perhaps solubilised, and may be subsequently washed away during the acetone extraction process which was performed prior to the chemical composition analysis. Similar solid loss during alkaline pretreatment has been reported by Nlewem and Thrash (2010) and Xu et al. (2009). Kashaninejad and Tabil (2011) investigated the effect of microwave and microwave-chemical pretreatments on barley straw grinds (1.58 mm screen size) using 1 and 2% NaOH concentration. The chemical composition analysis showed that microwave and chemical pretreatment significantly disintegrated the lignocellulosic

structure of barley straw grinds. The authors reported that there was about 45% and 62% lignin loss after 1% and 2% alkaline pretreatment, respectively. Varga et al. (2002) reported that 1%, 5%, and 10% NaOH treatments reduced lignin content in the solid fraction by 91.4%, 93.7%, and 95.9%, respectively. Millett et al. (1975) reported that alkali treatments have been applied in upgrading the nutritive value of forage and forest residues for ruminants. These authors stated that in general, soaking straw in about 1.5% sodium hydroxide for 24 h increases its digestibility from an initial 30 to 50% up to 60 to 70%.

This current investigation showed that the use of NaOH solution increased the ash content of the pretreated biomass (Table 3.4b). The ash content increased with the decreasing ratio of biomass to NaOH solution. This is in agreement with the findings of Rai and Mudgal (1987), who investigated the effect of NaOH on wheat straw at six concentrations; 0, 3, 5, 7, 9, and 12% (w/w). A similar trend in ash content was also observed by Kashaninejad and Tabil (2011) using 1 and 2% NaOH in microwave pretreatment of barley and wheat straw. In our results, 1% w/v NaOH concentration increased the ash content by about 60 to 140% depending on the ratio of biomass to NaOH solution. The ratio of biomass to NaOH solution and temperature have significant effects ($P<0.05$) on the ash content (Table 3.7). Ash content is a measure of the mineral content and other inorganic matter, structural or extractable in biomass (Sluiter et al. 2008). The lower the ratio of biomass to the NaOH solution, the higher is the ash content, due to the high concentration of mineral content of sodium in the NaOH solution at lower ratio. This problem of increased ash content can be addressed by washing the pretreated samples. Washing the pretreated samples reduced the ash content by about 25-55% depending on the ratio of biomass to the NaOH solution. It was also observed that the washed samples have lower acid insoluble lignin and soluble lignin moieties. This may be due to loss of solid lignin and the solubilised lignin during the washing process.

Table 3.4a Chemical composition (% dry basis) analysis of radio frequency alkaline pretreated and non-treated barley straw grind

Chemical compositions	Temp (ºC)	NT	Biomass: NaOH Solution Ratio				
			1:4	1:5	1:6	1:7	1:8
Acid Insoluble Lignin (%)	24	20.12 (0.06)	-	-	21.57 (3.91)	17.99 (0.08)	-
	70	-	19.56 (0.56)	17.8 (0.28)	17.91 (0.21)	17.82 (0.55)	18.53 (0.17)
	80	-	19.22 (0.09)	18.22 (0.44)	18.64 (0.96)	18.10 (0.85)	18.86 (0.25)
	80^{W}	-	-	15.62 (0.30)	-	12.39 (0.55)	-
	80^{DW}	-	-	-	-	19.90 (0.14)	-
	80^{TW}	-	-	-	-	20.38 (0.18)	-
	90	-	20.66 (1.35)	17.64 (0.87)	18.38 (0.25)	18.05 (0.37)	19.27 (0.13)
	90^{W}	-	-	15.37 (0.30)	-	10.98 (0.12)	-
Acid Soluble Lignin using UV-Vis Spec. (%)	24	1.64 (0.10)	-	-	1.60 (0.03)	1.40 (0.15)	1.40 (0.02)
	70	-	1.38 (0.00)	1.40 (0.06)	1.57 (0.02)	1.63 (0.05)	1.43 (0.04)
	80	-	1.39 (0.09)	1.38 (0.06)	1.60 (0.05)	1.62 (0.05)	-
	80^{W}	-	-	1.37 (0.02)	-	1.39 (0.05)	-
	80^{DW}	-	-	-	-	1.66 (0.03)	-
	80^{TW}	-	-	-	-	1.65 (0.02)	-
	90	-	1.20 (0.27)	1.60 (0.04)	1.36 (0.07)	1.69 (0.01)	1.41 (0.09)
	90^{W}	-	-	1.32 (0.14)	-	1.27 (0.24)	-

Value in parentheses is standard error; n = 2; Temp = Temperature; NT = non-treated biomass;
W = washed; DW = distilled water; TW = tap water; 1% NaOH concentration was used.

Table 3.4b Chemical composition (% dry basis) analysis of radio frequency alkaline pretreated and non-treated barley straw grind

Chemical compositions	Temp (ºC)	NT	Biomass: NaOH Solution Ratio				
			1:4	1:5	1:6	1:7	1:8
Ash Content (%)	24	6.03 (0.36)	-	-	11.94 (0.00)	13.17 (0.19)	-
	70	-	9.51 (0.03)	10.93 (0.17)	11.57 (0.08)	13.25 (0.25)	14.11 (0.03)
	80	-	9.79 (0.25)	11.15 (0.07)	12.02 (0.11)	13.61 (0.31)	14.73 (0.24)
	80^{W}	-	-	3.94 (0.04)	-	2.69 (0.05)	-
	80^{DW}	-	-	-	-	5.93 (0.25)	-
	80^{TW}	-	-	-	-	6.08 (0.22)	-
	90	-	9.76 (0.03)	10.78 (0.09)	11.43 (0.02)	13.42 (0.11)	13.83 (0.03)
	90^{W}	-	-	4.37 (0.19)	-	2.63 (0.14)	-
Ash Increase/ Decrease (%)	24	0.00	-	-	98.11	118.60	-
	70	-	57.80	81.30	92.05	119.93	134.13
	80	-	62.51	85.01	99.53	125.80	144.37
	80^{W}	-	-	-34.63	-	-55.30	-
	80^{DW}	-	-	-	-	-1.56	-
	80^{TW}	-	-	-	-	0.93	-
	90	-	62.00	78.82	89.63	122.75	129.59
	90^{W}	-	-	-27.47	-	-56.28	-

Value in parentheses is standard error; n = 2; Temp = Temperature; NT = non-treated biomass;
W = washed; DW = distilled water; TW = tap water; 1% NaOH concentration was used.

Table 3.5 shows that the use of NaOH solution and decreasing the ratio of biomass to the NaOH solution increased the total acid soluble lignin moieties. This result reflects that the use of NaOH leads to pore creation within the lignin matrix (Nlewem and Thrash, 2010), which makes it easy to access the acid soluble lignin component during the chemical composition analysis (two-step acid hydrolysis). This investigation shows that the concentration of NaOH and the ratio of biomass to the NaOH solution are the dominant contributing factors, while temperature plays a lesser role. The heat provided by the RF is needed to assist the alkaline solution in the deconstruction and disaggregation of lignocellulosic biomass matrix.

The statistical analysis shows that the ratio of biomass to NaOH solution has significant effect on the total acid soluble lignin moieties measured using UPLC ($P<0.05$) (Table 3.6). The interaction between the ratio and temperature has no significant effect on the total acid soluble lignin moieties. However, the total acid soluble lignin moieties yield showed that RF-assisted alkaline pretreatment was effective at all the temperatures studied.

The lignin in the raw sample is more difficult to solubilise during the chemical composition analysis, because the lignified matrix is tightly bound to the other constituents. A biomass to NaOH solution ratio of 1:6 resulted in the maximal effect of pretreatment, with yields of the total acid soluble lignin moieties 31-64% greater than from the non-treated biomass.

Table 3.5 Total acid soluble lignin moieties (ng mg^{-1}) (dry basis) of radio frequency alkaline pretreated and non-treated barley straw grind.

Temp	NT	Biomass: NaOH Solution Ratio				
(^oC)		1:4	1:5	1:6	1:7	1:8
24	287 (68.8)	-	-	472 (43.4)	386 (10.7)	-
70	-	331 (46.8)	348 (4.7)	419 (57.2)	366 (43.8)	416 (63.3)
80	-	294 (13.4)	311 (6.7)	440 (34.4)	382 (38.6)	369 (6.64)
80^W	-	-	203 (3.3)	-	208 (2.39)	-
80^{DW}	-	-	-	-	246 (48.4)	-
80^{TW}	-	-	-	-	236 (0.33)	-
90	-	319 (36.3)	461 (20.8)	376 (83.5)	381 (42.5)	427 (22.6)
90^W	-	-	207 (10.7)	-	180 (7.12)	-

Value in parentheses is standard error; n = 2; Temp = temperature; NT = non-treated biomass;
W = washed; DW = distilled water; TW = tap water; 1% NaOH concentration was used.

Easy access to the total acid soluble lignin moieties is associated with the breakdown of the lignified biomass matrix, due to the already initiated pore swelling during the RF-assisted alkaline pretreatment process. This reflects maximum enhancement of the accessibility and digestibility of the cellulose and hemicellulose by 64% during the enzymatic reaction. This will also help in decreasing the costs and amount of enzymes required for the next stage of enzymatic hydrolysis by about 64% compared to the non-treated biomass. Nlewem and Thrash (2010) reported that alkaline pretreatment increases lignin solubilisation. The samples pretreated with tap or distilled water had lower acid solubilised lignin, which may be attributed to the hydrophobic nature of lignin. Therefore, for efficient breakdown and disruption of the lignin bonds or to create sufficient pores on the biomass matrix, the right ratio of biomass to NaOH solution and adequate uniform heat distribution are required. It was also observed from this investigation that biomass can be alkaline pretreated even at room temperature if the required ratio of biomass and NaOH solution is applied. Azzam (1989) investigated the pretreatment of cane bagasse with hydrogen peroxide and reported that hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% of the lignin and most of the hemicellulose were solubilised by 2% H_2O_2 at 30°C (warm room temperature) within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45°C for 24 h. The ratio of biomass to NaOH solution and temperature did not show any significant effect on the acid soluble lignin measured using the UV-Vis spectroscopy. This may be attributed to the capability of the UV-Vis spectroscopy. Washing the RF alkaline pretreated sample decreased the acid solubilised lignin content and tended to increase the furfural content.

Table 3.6 also lists the average sum of the monomeric reduced simple sugars (xylose, arabinose, glucose, galactose, and mannose) measured. Hemicellulose was calculated from the sum of xylose, arabinose, galactose, and mannose, while all of the measured glucose was assumed to be derived from cellulose.

Table 3.6 Furfural, cellulose, and hemicellulose contents (% dry basis) of radio frequency alkaline pretreated and non-treated barley straw grind.

Chemical compositions	Temp (ºC)	NT	Biomass: NaOH Solution Ratio				
			1:4	1:5	1:6	1:7	1:8
Average Furfural (%)	24	4.43 (0.18)	-	-	3.14 (0.11)	4.89 (1.84)	-
	70	-	3.51 (0.39)	3.85 (0.92)	4.29 (0.35)	5.34 (1.07)	3.66 (0.67)
	80	-	3.37 (0.40)	3.75 (1.46)	3.54 (0.18)	4.52 (1.55)	3.78 (0.70)
	80^{W}	-	-	5.80 (1.26)	-	6.29 (0.15)	-
	80^{DW}	-	-	-	-	5.57 (0.58)	-
	80^{TW}	-	-	-	-	5.17 (0.87)	-
	90	-	4.29 (0.81)	6.09 (1.15)	3.30 (0.75)	4.52 (0.14)	3.56 (1.44)
	90^{W}	-	-	5.25 (0.77)	-	5.16 (0.79)	-
Cellulose (%)	24	42.51(11.31)	-	-	30.93 (6.38)	25.78 (5.91)	-
	70	-	22.25 (1.70)	24.21 (1.56)	28.25 (6.23)	27.39 (6.32)	26.73 (1.77)
	80	-	22.37 (1.45)	21.07 (4.34)	31.94 (2.71)	26.08 (4.72)	18.44 (3.60)
	80^{W}	-	-	27.69 (6.81)	-	38.20 (3.52)	-
	80^{DW}	-	-	-	-	21.88 (1.61)	-
	80^{TW}	-	-	-	-	24.98 (2.82)	-
	90	-	26.93 (1.00)	24.65 (7.94)	30.37 (3.36)	21.33 (0.62)	22.68 (2.66)
	90^{W}	-	-	33.44 (8.70)	-	33.31 (11.90)	-
Hemicellulose (%)	24	29.98 (6.60)	-	-	29.12 (1.51)	23.40 (0.87)	-
	70	-	23.00 (0.33)	21.63 (0.49)	23.18 (0.25)	24.27 (0.46)	26.76 (3.01)
	80	-	22.14 (0.41)	21.38 (5.73)	26.14 (5.61)	26.75 (0.30)	18.32 (2.71)
	80^{W}	-	-	21.60 (4.48)	-	27.72 (1.58)	-
	80^{DW}	-	-	-	-	19.12 (5.43)	-
	80^{TW}	-	-	-	-	21.04 (0.37)	-
	90	-	26.24 (3.39)	21.05 (4.61)	22.36 (0.53)	22.27 (5.23)	19.00 (3.10)
	90^{W}	-	-	26.08 (3.82)	-	20.69 (2.64)	-

Value in parentheses is standard error (n = 2); Temp = Temperature; NT = non-treated biomass;
W = washed; DW = distilled water; TW = tap water; 1% NaOH concentration was used.

The preserved cellulose from the raw sample (non-treated) is higher than that from the RF alkaline pretreated samples because of the initial degradation of the sugars during the pretreatment process. The same observation applies to hemicellulose. This implies that there is a trade-off between the breakdown of the biomass matrix/creating pores in the lignin and enhancing the accessibility and digestibility of the cellulose and hemicellulose. The more the biomass matrix (lignin bond) is broken down, the more the components of interest (cellulose and hemicellulose) were degraded. Varga et al. (2002) reported that 1%, 5%, and 10% NaOH treatments reduced hemicellulose in the solid fraction by 65.9%, 79.2%, and 88.2%, respectively; and reduced cellulose by 30.8%, 41.9%, and 53.3%, respectively. The stronger alkaline pretreatment caused more solubilisation of cellulose and hemicellulose. Kashaninejad and Tabil (2011) reported that there was reduction of cellulose and hemicellulose after microwave alkaline pretreatment, with 2% NaOH causing more reduction than 1% concentration. Millett et al. (1975) reported that one of the disadvantages of the pretreatment of straws for ruminant feed is that there is considerable solubilisation of hemicellulose. In our investigation, the ratio of biomass to NaOH solution has significant effect on cellulose and hemicellulose ($P<0.05$), as shown on Table 3.7. There was no significant effect on both components from the interaction between ratio and temperature. In general, the treatment combinations using the biomass to NaOH solution ratio of 1:6 at all temperatures studied resulted in optimum yields of the cellulose and hemicellulose and total acid soluble lignin moieties. At optimal conditions, pretreatment enhanced hemicellulose yields by 75-97%, compared to enhancements of 67-75% yield for cellulose. Hemicellulose had higher yield because of its random, branched, and amorphous structure with little strength unlike the crystalline, highly ordered cellulose with a high degree of polymerisation that requires more severe pretreatment conditions.

Wang et al. (2008) evaluated the effect of alkaline pretreatment of coastal Bermuda grass for bioethanol production at 121°C using 1%, 2%, and 3% (w/v) NaOH for 15, 30, 60, and 90 min. They reported that pretreatment with 1% (w/v) NaOH concentration yielded significantly more reducing sugars than 2% and 3%; higher NaOH concentrations resulted in higher total solid loss (acid insoluble lignin). They reported that degradation of the reducing sugars occurred during the alkaline pretreatment, which led to decreased cellulose and hemicellulose. It was also reported

that with the increase of pretreatment severity, more lignin and hemicellulose were dissolved into the NaOH solution, while cellulose was well-preserved due to its semicrystalline structure (Wang 2008). Kumar et al. (2009) reported that hemicellulose can be readily hydrolysed by dilute acids under moderate conditions, but much more extreme conditions are needed for cellulose hydrolysis. In our current investigation, pretreatment at room temperature with biomass to NaOH solution ratio of 1:6 resulted in good yield of the monosaccharides. This is an indication that the use of NaOH solution and the required ratio of biomass to NaOH solution are the major contributors to the enhancement of the accessibility and digestibility of the energy potentials for the biofuel industry. Ramesh and Singh (1993) reported that barley straw theoretically contains about 40% cellulose, 20% hemicellulose, and 15% lignin. Marsden and Gray (1985) also reported that barley straw theoretically contains about 44% cellulose, 27% hemicellulose, and 7% lignin. These values are comparable with the values obtained from the non-treated sample in this investigation. It should be noted that the variance in the chemical composition between the reported theoretical values and the values obtained from this investigation may be attributed to differences in locations where the crop was grown, weather conditions, the barley variety grown, and different methods of analysis. The National Renewable Energy Laboratory protocol used for the chemical composition analysis (two-step acid hydrolysis) could also contribute to the degradation of the already released energy potential because of the harsh conditions of acid used (72% H_2SO_4). Washing the pretreated samples seemed to improve the UPLC detection of the monomeric sugars. The washed samples had higher percentage of cellulose and hemicellulose, indicating that the NaOH present in the unwashed RF alkaline pretreated sample may have interfered with the detection power and performance of the UPLC, or possible reaction between NaOH and the chemical solutions used during the two-step acid hydrolysis. No statistical difference was observed for furfural.

3.5.4 Comparison with Steam Explosion and Microwave-Alkaline Pretreatment

Adapa et al. (2011b) applied steam explosion for the pretreatment of ground barley straw, with 30 mm hammer mill screen size, performed using a pilot-scale continuous steam explosion plant. The authors reported that 25.3% cellulose, 21.0% hemicellulose, 1.4% soluble lignin, and 20.2%

insoluble lignin were obtained from the pretreatment based on percentage dry basis. In our studies, at the optimal pretreatment conditions (ratio 1:6, 24-90^{o}C), 28.2-31.9% cellulose, 22.4-29.1% hemicellulose, 1.4-1.6% soluble lignin, and 17.9-21.6% insoluble lignin were obtained (Tables 3.4-3.6). This implies that RF-assisted alkaline pretreatment is more efficient than steam explosion pretreatment. RF-assisted alkaline pretreatment results to higher accessibility of the energy potential (cellulose and hemicellulose) and soluble lignin, with higher degradation of insoluble lignin than the steam explosion method. However our current investigation led to increase in the ash content of the pretreated biomass.

Kashaninejad and Tabil (2011) investigated the effect of microwave and microwave-chemical pretreatments on barley straw grinds with hammer mill screen opening size of 1.58 mm. The authors reported that 40.81% cellulose, 8.74% hemicellulose, and 6.65% lignin were obtained based on percentage dry basis at 713 W power level. Less cellulose was released in the RF assisted-alkaline pretreatment in our current studies; however, more hemicellulose was released in the RF assisted-alkaline pretreatment than the microwave alkaline pretreatment. The differences in the obtained chemical compositions may be attributed to the different standards used for the chemical composition analysis and feedstocks variance. The energy consumption for the microwave chemical pretreatment was not recorded, but by principle and mechanism of operation, it can be assumed that the energy consumption in both the RF-assisted alkaline and microwave alkaline pretreatments will be comparable. Due to the low power level of the RF machine, the microwave alkaline pretreatment takes less time to heat up to the preset temperature. However, the uniform heat distribution, heat penetration depth, and avoidance of thermal run away associated with RF heating are the added advantages over the microwave application that need to be considered for scale up purposes.

Table 3.7 Analysis of variance determining the effect of independent variables (temperature, biomass:NaOH solution ratio, and their interaction) on the dependent variable (acid soluble lignin, cellulose, hemicellulose, ash content, and furfural).

Dependent and independent variables	P-value
Acid Soluble Lignin	
Temperature	0.44
Biomass:NaOH Solution (Ratio)	<0.01
Ratio*Temperature	0.44
Cellulose	
Temperature	0.92
Biomass:NaOH Solution (Ratio)	0.02
Ratio*Temperature	0.88
Hemicellulose	
Temperature	0.71
Biomass:NaOH Solution (Ratio)	0.04
Ratio*Temperature	0.49
Ash content	
Temperature	<0.01
Biomass:NaOH Solution (Ratio)	<0.01
Ratio*Temperature	<0.01
Furfural	
Temperature	0.81
Biomass:NaOH Solution (Ratio)	0.52
Ratio*Temperature	0.84

3.6 Conclusion

The applicability of radio frequency heating in the alkaline pretreatment method of lignocellulosic biomass has been investigated. We conclude that, in comparison with other pretreatment technologies such as steam explosion, RF alkaline pretreatment usually involves lower temperatures and pressures. RF heating generates uniform heat distribution with no local hot spots or thermal runaway unlike the microwave heating. However, RF alkaline pretreatment involves longer pretreatment time with the low power level (1.5 kW) of the RF setup used in this study. The pretreatment time of this combined approach (RF-assisted alkaline pretreatment) using higher power RF will be shorter. The use of NaOH solution and the ratio of biomass to NaOH solution played a more important role than temperature during the RF alkaline pretreatment. It may also be possible that the use of these variables (NaOH solution and the ratio of biomass to NaOH solution) is more important than the method of heating. In other words,

using a different method of heating (other than RF approach) may also reflect the major role of the above mentioned variables. All ratios of biomass to NaOH solution that we tested (compared to no NaOH treatment) had the following effects on the biomass: more lignin was structurally disrupted and released; lower acid insoluble lignin and higher total acid soluble lignin moieties were produced; the accessibility and digestibility of the cellulose and hemicellulose was increased, leading to greater cellulose and hemicellulose degradation; the ash content was increased; and required higher energy consumption and longer pretreatment time.

This implies that during alkaline pretreatment, there is a trade-off between the breakdown of lignin bonds and carbohydrate degradation. If the acid soluble lignin is easily accessed, it will help in the easy accessibility and digestibility of the carbohydrates during the next stage of the bio-ethanol production (enzymatic hydrolysis). Subsequently, this will assist in decreasing the required amount and costs of enzymes by up to 64% compared to the non-treated biomass. This improvement is connected with lignin removal. Washing of the RF-assisted alkaline pretreated samples reduced the ash content. Based on this study, it was observed that: biomass intended to be used for bio-ethanol production does not necessarily require high and extreme conditions for the pretreatment process. This will help to avoid the degradation of the cellulose and hemicellulose. Water and heat are not sufficient to disrupt the lignin bonds or create pores on the biomass matrix. RF assisted-alkaline pretreatment technique represents an easy to set-up and potentially affordable route for the bio-fuel industry, but this requires further energy analysis and economic validation, so as to investigate the significant high energy consumption during the RF-assisted alkaline pretreatment heating process.

Chapter 4

4. Pretreatment and Fractionation of Barley Straw using Steam Explosion at Low Severity Factor

A similar version of this chapter has been published in the journal of Biomass and Bioenergy:

- Iroba, K.L., L.G. Tabil, S. Sokhansanj and T. Dumonceaux. 2014. Pretreatment and fractionation of barley straw using steam explosion at low severity factor. *Biomass and Bioenergy* 66: 286-300.

Contributions of Ph.D. Candidate

The experimental design, data analysis and manuscript writing were performed by Kingsley Iroba while Dr. Lope Tabil, Dr. So*khansanj and Dr.* Dumonceaux provided editorial input. In addition, Dr. Lope Tabil established research collaboration with the Clean Energy Research Center, University of British of Columbia, Vancouver, BC, Canada where the steam explosion experiment was conducted. The chemical compositions analysis was performed at the Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatchewan Canada.

Contribution of this Paper to Overall Study

Knowledge Gap: Perusal of literature shows that steam explosion on lignocellusic biomass has always been performed at higher temperature of 200-260°C with a corresponding saturated pressure of 2000-5000 kPa. Similarly, the focus of steam explosion on lignocellulosic biomass has always been on the effects of temperature, retention time, biomass screen size, and pH. However, little or no work has been done on effects of feedstock initial moisture content on the

severity of the steam explosion technologies. Therefore, the objective of this current investigation was to assess the operating conditions for steam explosion treatment of biomass straw at low severity factor and conditions with minimal production of inhibitors (furfural) and to further evaluate the process as the biomass substrate is affected by the initial moisture content of the biomass, temperature (or pressure) of steam, and retention time of the treated biomass in the steam reactor.

Justification: The biodegradation via enzymatic hydrolysis of native lignocellulosic biomass usually results in low yield of simple sugars, hence initial pretreatment is required. Chornet and Overend (1998) reported that the severity of thermal pretreatment depends on the combined effects of temperature and moisture particularly in the amorphous compounds in biomass. In the present study, it is hypothesized that using higher biomass initial moisture content and lower temperature could damp the severe steam explosion pretreatment conditions and lead to reduction in the degradation of the xylose and glucose. Cullis et al. (2004) studied the effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. It was reported that moisture content plays an influential role in the severity of pretreatment, as it greatly influences the ability of heat and chemicals to penetrate wood.

4.1 Abstract

Agricultural residues represent an abundant, readily available, and inexpensive source of renewable lignocellulosic biomass. However, biomass has complex structural formation that binds cellulose and hemicellulose. This necessitates the initial breakdown of the lignocellulosic matrix. Steam explosion pretreatment was performed on barley straw grind to assist in the deconstruction and disaggregation of the matrix, so as to have access to the cellulose and hemicellulose. The following process and material variables were used: temperature (140-180^{o}C), corresponding saturated pressure (500-1100 kPa), retention time (5-10 min), and mass fraction of water 8-50%. The effect of the pretreatment was assessed through chemical composition analysis. The severity factor R_o, which combines the temperature and time of the hydrolytic process into a single reaction ordinate was determined. To further provide detailed chemical composition of the steam exploded and non-treated biomass, ultimate analysis was

performed to quantify the elemental components. Data show that steam explosion resulted in the breakdown of biomass matrix with increase in acid soluble lignin. However, there was a considerable thermal degradation of cellulose and hemicellulose with increase in acid insoluble lignin content. The high degradation of the hemicellulose can be accounted for by its amorphous nature which is easily disrupted by external influences unlike the well-arranged crystalline cellulose. The carbon content of the solid steam exploded product increased at higher temperature and longer residence time, while the hydrogen and oxygen content decreased, and the higher heating value (HHV) increased.

4.2 Introduction

The depleting fossil fuel resources along with global warming associated with greenhouse gas (GHG) emission has been a serious concern to the global community. Presently, this has necessitated looking for a renewable, sustainable, and environmentally friendly energy. Lignocellulosic biomass has been identified as one of the renewable, near carbon neutral, and local fuel sources to complement fossil fuels and to contribute to energy security (Zhang et al. 2007). It is estimated that the worldwide annual production of lignocellulosic biomass (agricultural and forest resources) is approximately 200 Gt (Zhang et al. 2007). However, there are some challenges associated with the use of lignocellulosic biomass. Lignocellulosic biomass is a complex formation of cellulose, hemicellulose, and lignin. The lignin acts as an external crosslink binding the hemicellulose and cellulose with cellulose positioned at the inner core of the structure (Fan et al. 2006; Lin and Tanaka 2006). This nature of lignocellulosic biomass matrix makes it naturally resistant to the microbial and enzymatic degradation needed for biochemical conversion. Hence, the goal for the biofuel industry is to make the process technically feasible and economically attractive. Many pretreatment technologies have been applied in the past decades to deconstruct and fractionate a complex lignocellulose structure to its simpler molecules.

Canada has a large agricultural sector with barley production as the second largest crop behind wheat (FAOSTAT 2007). This implies that Canada will play a very significant role in the development of the biorefinery sector because of its contribution as a predominant supplier or

source of biomass feedstock. Barley straw has a higher mass fraction of cellulose (40%) than wheat straw (30%), although wheat straw has a higher mass fraction of hemicellulose (50%) than barley straw (20%), with both substrates having similar mass fraction of lignin (15%) (Ramesh and Singh 1993; Jørgensen et al. 2007). Perusal of the literature shows that a lot of research has been done on wheat straw; therefore, there is a need to explore the potential of barley straw for the biofuel industries. Hence, for these reasons as well as its higher cellulose content than wheat straw, barley straw was chosen for this work.

4.2.1 Steam Explosion

Steam explosion is operated by introducing the feedstock into the reactor and heating under steam pressure (2000-5000 kPa; 200-260°C) for a few minutes (Toussaint et al. 1991). Steam explosion induces chemical effects because "water itself acts as an acid at high temperatures" (Mosier et al. 2005). In the course of the process, hemicellulose is hydrolyzed by acetic acid with other acids released, and the hemicellulose eventually becomes water-soluble (Toussaint et al. 1991; Mosier et al. 2005). Cellulose is mildly depolymerized, lignin softens and is eventually depolymerized. The reaction is instantaneously interrupted by sudden opening of the reactor to depressurize the treated material which is discharged through a nozzle into a collection vessel (Toussaint et al. 1991). The sudden decompression rapidly decreases the temperature and suppresses the reaction at the end of the pretreatment process. The sudden thermal expansion involved in the termination of the reaction causes the particulate structure of the biomass to open up (Mosier et al. 2005). However, at excessive conditions (high temperatures and pressures), there is degradation of xylose (a hemicellulose) to furfural and glucose (a cellulose monomer) to 5-hydroxymethyl furfural (Toussaint et al. 1991; Tanahashi et al. 1998). Furfural is an undesirable compound in a fermentation process because it inhibits microbial growth and activity (Toussaint et al. 1991; Tanahashi et al. 1998). Chornet and Overend (1998) reported that the severity of thermal pretreatment depends on the combined effects of temperature and moisture particularly in the amorphous compounds in biomass. In the present study, we hypothesized that using higher biomass initial moisture content and lower temperature could damp the severe steam explosion pretreatment conditions and lead to reduction in the degradation of the xylose and glucose. Chemical consequences of thermal treatment of lignocellulosics either in a

suspension (large amount of liquids present) or in steaming processes (low amount of free water present) was summarized by Chornet and Overend (1998) as follows: "the extent of disaggregation of lignin-carbohydrates complexes is a function of the severity of the treatment, and the degree of depolymerization of hemicellulose and cellulose polymers and the rupture of glycosidic bonds".

4.2.2 Severity Factor

Due to the different types of available lignocellulosic biomass, a severity factor has been developed to combine and normalize the process parameters of steam explosion so as to facilitate comparisons (Chornet and Overend 1998). Effort has been in progress to minimize product degradation resulting from pretreatment conditions. The rate constant of the severity factor, according to Chornet and Overend (1998) can be described by the Arrhenius model:

$$k = Ae^{-\frac{Ea}{RT}} \tag{4.1}$$

k = rate constant; A = Arrhenius frequency factor; Ea = activation energy (kJ kg^{-1} mol^{-1})

R = universal gas constant (8.314 kJ kg^{-1} mol^{-1} K^{-1}); T = absolute temperature (K).

The reaction ordinate was developed as:

$$R_o = \int_0^t \exp\left[\frac{(T_r - T_b)}{14.75}\right] dt \tag{4.2}$$

R_o = reaction ordinate; t = residence time (min); T_r = reaction temperature (^oC);

T_b = base temperature at 100^oC (above which biomass compositions start to change); (14.75 is the conventional energy of activation assuming that the overall process is hydrolytic and the overall conversion is first order), upon derivation of equation 4.3 from equation 4.2, T_r was assumed to be constant, therefore;

$$R_o = t\ \exp\left[\frac{(T_r - 100)}{14.75}\right] \tag{4.3}$$

The log value of the reaction ordinate yields the severity factor which is used to analyze the effects of steam explosion pretreatment on lignocellulosic biomass.

$$Severity = log_{10}(R_o) \tag{4.4}$$

Heitz et al. (1991) reported that it is possible to directly translate results obtained from laboratory steam explosion pretreatment processes into industrial practice by employing the concept of a severity factor. This is similar to those used in pulping industry which integrates temperature and retention time into a single measure for the purpose of controlling the process (Chundawat and Venkatesh 2006).

Chum et al. (1990) investigated the effect of acid catalyzed pretreatment on aspen (*Populus tremuloides*). These authors included a pH term to equation (4.7) above to compensate for the contribution of the added acid. Severity parameter that reflects acidity or alkalinity functions can be very useful tool in the planning of experiments for pretreatment process that requires the addition of catalyst (acid/base) (Chum et al. (1990). However, it was concluded that the reaction ordinate concept does not appear to be universally valid (Kaar et al. 1998). Nonetheless, the reaction ordinate remains a useful bookkeeping method of reporting steam explosion conditions.

4.2.3 Heating Value of Biomass

The heating value of biomass is a key element for the planning, design and control of biomass power plants. The heating (calorific) value of lignocellulosic biomass feedstocks is a representative of the energy content they possess as potential biofuel. "The gross calorific value (higher heating value, HHV) and the net calorific value (lower heating value, LHV) at constant pressure" condition measure the enthalpy change of combustion with and without water condensation, respectively (Sokhansanj 2011; Demirbas 2007; Annamalai et al. 1987). The heating values of biomass materials can be determined experimentally using an adiabatic bomb calorimeter or they can be estimated by using mathematical models based on the chemical composition, proximate or ultimate analysis of the biomass fuel (Sheng and Azevedo 2005; Yin et al. 2011). The theoretical HHV of barley straw has been reported as 17.31(MJ kg^{-1}, dry basis) by Annamalai et al. (1987).

Previous studies done on the optimization of steam explosion of lignocellulosic biomass focused on the effects of steam explosion temperature, retention time, biomass screen size, and pH. However, little or no work has been done on effects of feedstock initial moisture content on the

severity of the steam explosion technologies. Consequently, the objective of this current investigation was to study steam explosion pretreatment of barley straw as the biomass substrate is affected by the initial moisture content of the biomass, temperature (or pressure) of steam, and retention time of the treated biomass in the steam reactor.

4.3 Materials and Methods

4.3.1 Sample Collection and Preparation

Barley straw of the 'Xena' variety was obtained in October, 2009 from RAW Ag Ventures Limited (Maymont, SK, Canada), and was grown at 52.667297º N and 107.794311º W. The straw was ground using a hammer mill (Serial no. GM13688; Glen Mills Inc., Maywood, NJ, USA) with screen size of 1.6 mm to increase the surface area of the biomass. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK, Canada) was connected to the outlet of the hammer mill to provide flow of the biomass in and out of the hammer mill and to control dust during operation. The initial mass fraction of water in the straw was 8.09%. The moisture content was measured based on ASABE standard method, ASAE S358.2 (2008). This test was performed in replicates of three. For details of the measurement, calculations, and results of the physical characteristics of the barley straw grind, see Iroba et al. (2013).

The samples were re-conditioned to the desired moisture content by spraying a pre-calculated amount of water on the samples to targeted moisture of the biomass, mixed manually and kept in a conditioned environment (~6^{o}C) for 48 h before the pretreatment process. Three process parameters were investigated for the optimization of steam explosion process. These variables (temperature (with corresponding saturated pressure), moisture content, and retention time) were investigated to determine the optimum condition for steam explosion pretreatment of biomass barley straw grind with minimal formation of inhibitors, and also to enhance formation of biomass pellets with good physical quality characteristics which was discussed in Iroba et al. (2014). The experimental design was based on a completely randomized approach, which resulted in 18 treatment combinations. Table 4.1 lists the levels of the independent variables.

Table 4.1 Independent variables with corresponding levels

Variables	Levels
Temperature (^{o}C)	140, 160, 180
Moisture content (% mass fraction)	8, 30, 50
Retention time (min.)	5, 10

A temperature range of 140-180^{o}C was selected so as to pretreat the biomass within the softening temperature of lignin and avoid or limit the thermal degradation of cellulose and hemicellulose and lignin. Fengel and Wegener (1984) reported that the softening temperature of lignin is in the range of 130-190^{o}C. The thermal stability of probable linkages between lignin and polysaccharides was compromised at temperatures above 200^{o}C (Fengel and Wegener 1984). Thermal degradation of lignin starts at 270-280^{o}C (Fengel and Wegener 1984).

4.3.2 Steam Explosion Process Description and Operation Procedures

The steam explosion pretreatment set up used for this investigation is located at the Clean Energy Research Center, University of British of Columbia, Vancouver, BC, Canada. Figure 4.1 below shows the process flow sheet of the closed system steam explosion batch unit.

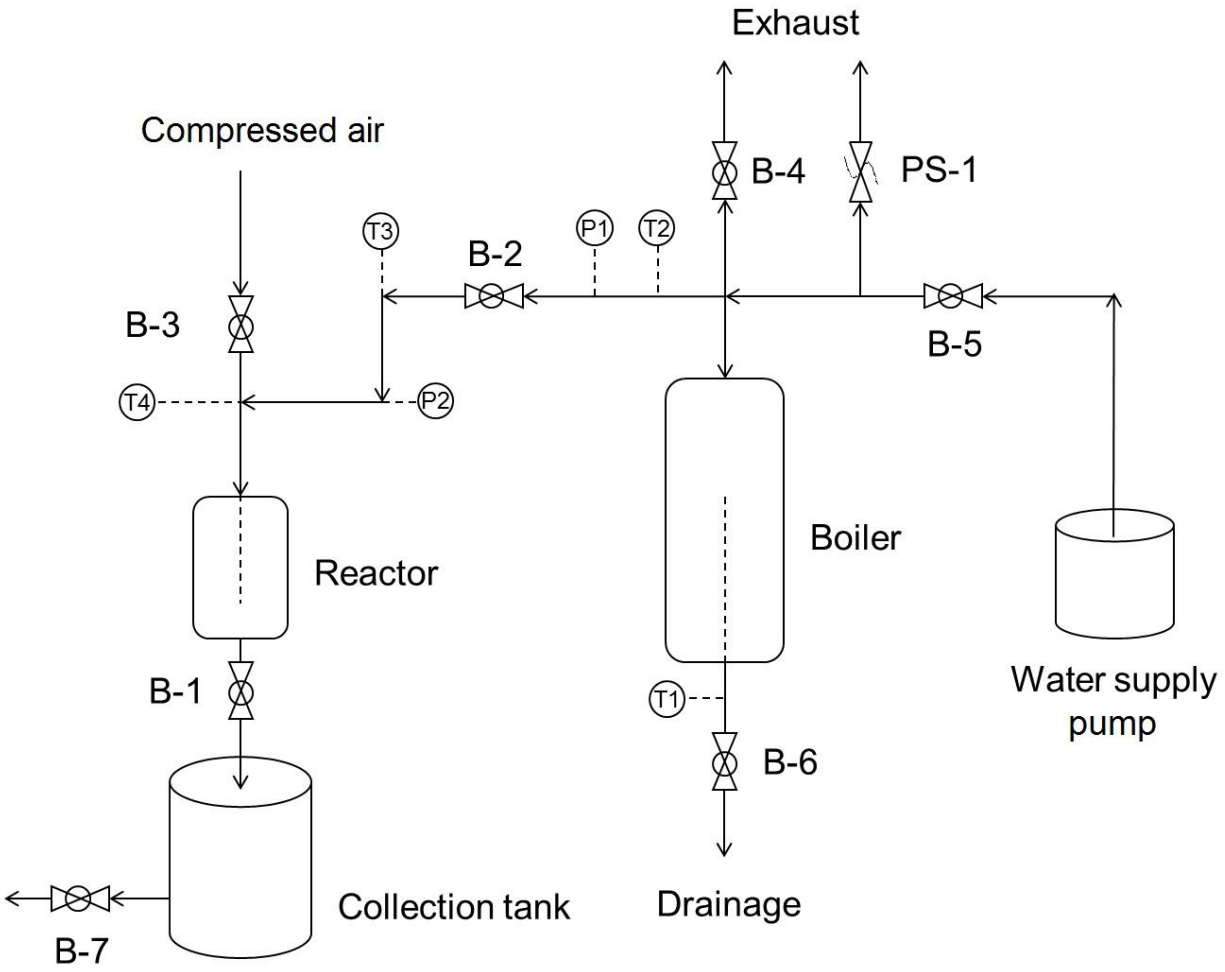


Figure 4.1 Process flow sheet of the closed system steam explosion unit (B: Ball valve, PS: Pressure relief valve, P: Digital pressure transducer, T: Thermocouple) (Lam 2011).

The unit consists of a 2 L steam generator, which generates saturated steam, and a 1 L reactor for steaming lignocellulosic biomass feedstocks. A 3 zone tubular furnace (Lindberg/Blue M, STF55666C, Thermo Fisher Scientific Inc., Waltham, MA, USA) installed inside the boiler chamber was used for the generation of the steam heat. The 1 L reactor was equipped with a 12.7 mm diameter ball valve (B-1), controlled by an electrical actuator for sudden discharge of the treated biomass into the collection vessel at ambient pressure. The service and maintenance heat of the reactor and the steam-line were regulated by a temperature controller (T1 and T4). The details and functions of valves in the experimental set-up unit are summarized elsewhere (Lam

2011). Temperatures and pressures were measured by 1.6 mm diameter K-type thermocouples, T1-T4 (Omega, Stamford, CT, USA) and digital pressure transducers, P1-P2 (Omega, Stamford, CT, USA), respectively. Data were collected by LabView 8.2 software (National Instruments, Austin, TX, USA).

4.3.2.1 Steam Generation

To generate the saturated steam required for the explosion in each experiment, about 350 cm^3 of distilled water was pumped from a water tank into the steam generator using a water pump. Prior to pumping the water, ball valves B-4 and B-6 were opened to purge the left over water in the steam boiler from the previous experiment. Any trapped air within the system was vented out by opening of B-4 valve which facilitated faster water drainage. These valves were closed after the water discharge. Ball valve B-5 was opened during the water pumping and closed thereafter. The actual water used for the steam generation was calculated from the difference between the loaded and the discharged water. Approximately 50 cm^3 of water was used as steam for each run of experiment. Ball valve (B-2) always remained closed during the purging and pumping process. Prior to addition of the barley straw grind into the reactor, ball valve (B-1) was closed so as to contain the loaded biomass within the reactor. Biomass of about 30-28 g was loaded through the small opening (about 10 mm) at the top of the reactor. Thereafter, ball valve B-3 (closed) was connected to the opening. Ball valve B-3 was also connected to compressed gas on the other end so as to facilitate the discharge of the treated sample.

4.3.2.2 Steam Explosion Experimental Runs

After adding biomass into the reactor (at room temperature and initial reactor pressure of approximately about 14 kPa) and pumping water into the steam generator, the temperature regulator of the steam boiler was turned on and set to a preset temperature (140-180^oC, depending on the treatment combination). It took between 30 and 50 min to reach the preset temperature depending on the treatment combinations. When the temperature reached regulated temperature (40^oC away from the preset temperature), the reactor temperature controller was turned on (Point 1 in Figures 4.2 and 4.3) and set to the same preset temperature as the steam boiler. This maintained thermal equilibrium and avoided heat loss from the steam to the reactor

as well as preventing condensation within the reactor. The target was for the reactor temperature and the generated steam temperature to get to the preset temperature (140-180^{o}C depending on the treatment combinations) simultaneously. When the temperature of the boiler reached the preset temperature with a corresponding saturated pressure, ball valve B-2 was opened (point 2 in Figures 4.2 and 4.3; for about 2-3 min depending on the treatment combinations) to transport the generated steam from the boiler to the reactor to treat the biomass for a pre-specified time. The generated steam was supplied to the reactor through the small opening (about 10 mm) at the top of the reactor. This led to a decrease in the boiler pressure and an increase in the reactor pressure as shown in Figure 4.3. This valve was closed when the same pressure (ranges from 500 to 1100 kPa depending on the treatment combinations) was observed in the boiler and the reactor. After the elapsed retention time (Points 3-4 in Figures 4.2 and 4.3), ball valve B-1 was opened (point 4 in Figures 4.2 and 4.3) to suddenly decompress the system, and the treated sample was discharged into a bag in the collection tank at ambient conditions (101 kPa, 23^{o}C). The collected steam exploded solid biomass product was weighed to determine the mass loss or gain and kept in sealed Ziploc containers at 6^{o}C for further analysis. Each test was replicated twice.

4.3.3.3 Data Acquisition and Control

Data acquisition and logging in this experiment were done using LabView 8.2 software (National Instruments, Austin, TX, USA). The data logging was one data point per second. The ball valve (B-1) used for the sudden decompression and opening of the bottom outlet of the reactor (to discharge the steam treated biomass) was controlled by Labview software.

Due to the small inlet opening (about 10 mm) as well as the moist nature of the samples, loading the samples into the reactor was very difficult. As such, a thin rod was used to push down the samples as they were loaded into the reactor. This resulted in the loaded samples sticking to the walls of the reactor. Since the heat source of the reactor was wrapped round it, the heat transfer was by conduction which comes from outside to inside of the reactor. This resulted in the wall of the reactor having a higher temperature than the inside of the reactor.

4.3.3 Chemical Composition Analysis

The chemical composition analysis was performed using the National Renewable Energy Laboratory standard (NREL) (Sluiter et al. 2007). The NREL standard uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The first step uses 72% H_2SO_4, while the second step uses 4% H_2SO_4.

Chemical composition analysis was done on the structural carbohydrates and lignin of the steam exploded and non-treated biomass. The lignin fractionates into acid insoluble and acid soluble material. The acid insoluble lignin (AIL) is the residue (remaining solids) from the hydrolysis suspension. The acid soluble lignin (ASL) was measured using two different NREL standard approaches; the first was using UV-Vis spectroscopy (Helios Aquamate v7.09 spectrophotometer, Thermo-Scientific, Cambridge, England). The second approach for measuring the ASL was done using the Ultra Performance Liquid Chromatography (UPLC) (Acquity 2004-2010, Waters Corp., Milford, MA, USA) which has the capability of separating and quantifying the various lignin components

During the two-step acid hydrolysis, the polymeric carbohydrates (cellulose and hemicellulose) were hydrolyzed into monomeric forms (xylose, arabinose, mannose, glucose, and galactose), which are soluble in the hydrolysis liquid. These were measured by UPLC. The furfurals: 1) 5-hydroxyl-methyl furfural; and 2) furfural, and the lignin moieties (components): 1) H acid lignin group (4-hydroxybenzoinc acid); 2) G acid lignin group (vanillic acid); 3) S acid lignin group (syringic acid); 4) H aldehyde lignin group (4-hydroxybenzaldehyde); 5) G aldehyde lignin group (vanillin); and 6) S aldehyde lignin group (syringaldehyde) were also measured using the UPLC. The spectra generated from the UPLC were integrated and extracted using Empower 2 software (Waters Corp., Milford, MA, USA). The extracted integrated peak area was correlated to the concentration of the standards using a pre-determined regression equation from dilution series using Microsoft Excel. Each test was replicated twice**.** For details on the chemical compositions analysis and regression see Iroba et al. (2013).

4.3.4 Determination of Ash in Biomass

The ash content, which is a measure of the mineral content and other inorganic matter (structural or extractable) in the biomass, was measured as part of the total composition. Structural ash, which is inorganic material, is bound in the physical structure of the biomass, while the extractable ash is the inorganic material that can be removed by washing or extracting the material (Sluiter et al. 2008). The ash content of the biomass samples was determined based on National Renewable Energy Laboratory standard (NREL) (Sluiter et al. 2008). About 0.5-2.0 g of the oven dried samples (dried @ 105^{o}C overnight) was weighed into a tared, dried crucible. The weighed crucible and the sample were placed in a muffle furnace (Model 650-14, Fisher Scientific, *Pittsburgh,* PA, USA) equipped with a thermostat with ramping program. Samples were heated to 105^{o}C, held at 105^{o}C for 12 min, ramped to 250^{o}C at 10^{o}C min^{-1}, held at 250^{o}C for 30 min, ramped to 575^{o}C at 20^{o}C min^{-1}, and held at 575^{o}C for 180 min. Then the temperature was decreased to 105^{o}C and held at 105^{o}C until the samples were removed. Each test was replicated twice and the ash content was computed as the percentage of residue remaining after the dry oxidation at 575^{o}C.

4.3.5 Ultimate Analysis of Steam Exploded and Non-Treated Lignocellulosic Biomass

In order to ascertain whether carbonization occurred during steam explosion process, the elemental composition analysis of the steam exploded and non-treated barley straw was performed. The measurement was done using the CHNS mode elemental analyzer vario EL III (Elementar Analysensteme GmbH, Serial No.: 11014041, Hanau, Germany). This set up is a fully automatic instrument for a speedy and quantitative determination of CHNS (Carbon, Hydrogen, Nitrogen, and Sulfur, respectively). Sulfanic acid (Sulfonilic acid) was used as standard for the system calibration. About 4.05-5.90 mg of the homogenized steam exploded and non-treated barley straw grind (0.354 mm screen size) carefully packed in tin foil (smooth wall tin capsules, Isomass Scientific Inc., elemental microanalysis, BN 182103) was combusted explosively in a highly oxygenated environment to produce the elements C, H, N, and S, in addition to the molecular nitrogen (N_2) and the oxidation products: O_2, H_2O, NO_X, SO_2, and SO_3. Each test was replicated twice.

4.3.6 Higher Heating Values

The HHV was experimentally measured using the oxygen bomb calorimeter (Parr Instrument Company, Model 1281, Moline, IL, USA). The procedure involves burning about 500-700 mg of pelleted sample in an oxygen-filled metal bomb submerged in a measured quantity of water, all held within a thermally insulated chamber. The exact rise in water-temperature resulting from combustion of sample was used to calculate the amount of heat units liberated, also referred to as gross energy or combustible energy, using equations 3.5-6 (Parr Instrument Company manual, Model 1281, Moline, IL, USA):

$$W = \frac{H x m + E_1 + E_2}{T_f - T_a} \tag{4.5}$$

W = Energy equivalent of calorimeter in MJ $^{o}C^{-1}$; H = Heat of combustion of standard benzoic acid in $MJkg^{-1}$; m = Mass of benzoic acid in kg; T_a = Temperature at the time of firing (^{o}C)

T_f = Final maximum temperature (^{o}C); E_1 = Correction for acid formation; E_2 = Correction for heat of combustion of fuse wire

$$H_g = \frac{[W x (T_f - T_a)] - (E_1 + E_2)}{weight\ of\ sample} \tag{4.6}$$

Where H_g = Gross heat of combustion of the sample in MJ kg^{-1}.

The mass yield and energy recovery were evaluated using the equations below according to Yan et al. (2009) and Ferro et al. (2004):

$$MY = \left(\frac{M_{SE}}{M_{NT}} \right) x100\% \tag{4.7}$$

$$ER = \frac{CV_{SE}\ x MY}{CV_{NT}} \tag{4.8}$$

where MY = mass yield, M_{SE} = mass of dried steam exploded solid, M_{NT} = Mass of dried non-treated solid, and ER = energy recovery, CV_{SE} is the calorific value of the steam exploded biomass product and CV_{NT} is the calorific value of the non-treated material

4.3.7 Degree of Carbonization (DOC)

The DOC was evaluated as:

$$DOC = \frac{CV_{SE}}{CV_{NT}} \tag{4.9}$$

where CV_{SE} is the calorific value of the steam exploded biomass product and CV_{NT} is the calorific value of the non-treated material.

4.3.8 Color Measurement

The color difference between the pretreated and non-treated was measured and compared using the HunterLab Color Analyzer (Hunter Associates Laboratory Inc, Reston, VA). The color was characterized using a three-dimensional color parameters (CIE L*, a* and b*). The scale L* ranges from 0 for black to +100 for white. The a* and b* have no specific numerical limits; positive a* is red; negative a* is green; positive b* is yellow; negative b* is blue. The total color difference (ΔE*) was also computed using equation 3.10 HunterLab 2008 (Hunter Associates Laboratory, Inc. Reston, VA, USA). This single value takes into account the differences between the L*, a*, and b* of the pretreated and the control (non-treated) material. Three replicates were performed for each sample.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{4.10}$$

$\Delta L^* = L^*_{Treated} - L^*_{NT}$; $\Delta a^* = a^*_{Treated} - a^*_{NT}$; $\Delta b^* = b_{*Treated} - b^*_{NT}$. These parameters ΔL*, Δa*, and Δb* indicate how much the non-treated (NT) and the pretreated differ from one another in L*, a*, and b*.

4.3.9 Statistical Analysis

Experimental data were statistically analyzed via analysis of variance (ANOVA) using simple linear regression model. The ANOVA was conducted at 5% significance level using IBM SPSS Statistics (Superior Performing Statistical Software, version 20 for Windows, 2012; IBM, Armonk, NY).

4.4 Results and Discussion

Figures 4.2 and 4.3 show the typical temperature and pressure profiles, respectively of an experiment at 180^{o}C, 50% mass fraction of water, and 5 min retention time. The profiles depict good agreement between the reactor and steam boiler temperatures and pressures. Figure 4.2 shows that the heat source to the reactor caused a temperature difference between the outside (reactor body) and inside portion of the reactor. Point 1 in Figures 4.2 and 4.3 represent the time the reactor temperature was turned on, Point 2 shows the time ball valve B-2 was opened to transfer the generated steam from the boiler to the reactor. This led to a decrease in the boiler pressure and an increase in reactor pressure. Point 3-4 depict the preset retention time (5 min for this treatment combination), while point 4 is the time at which ball valve B-1 (at the outlet bottom of the reactor) was opened to suddenly decompress the system.

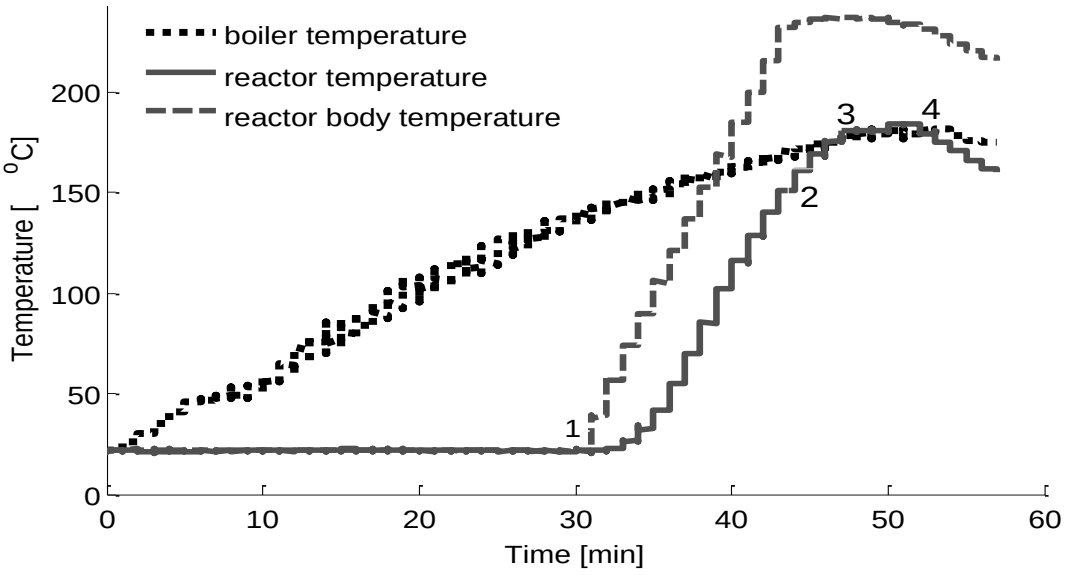


Figure 4.2 Typical temperature profile of an experiment (180^{o}C, 50% moisture content (w.b.), and 5 min retention time); Point 1 = reactor temperature was turn on; Point 2 = ball valve B-2 was opened; Point 3-4 = preset retention time; point 4 = ball valve B-1 was suddenly opened.

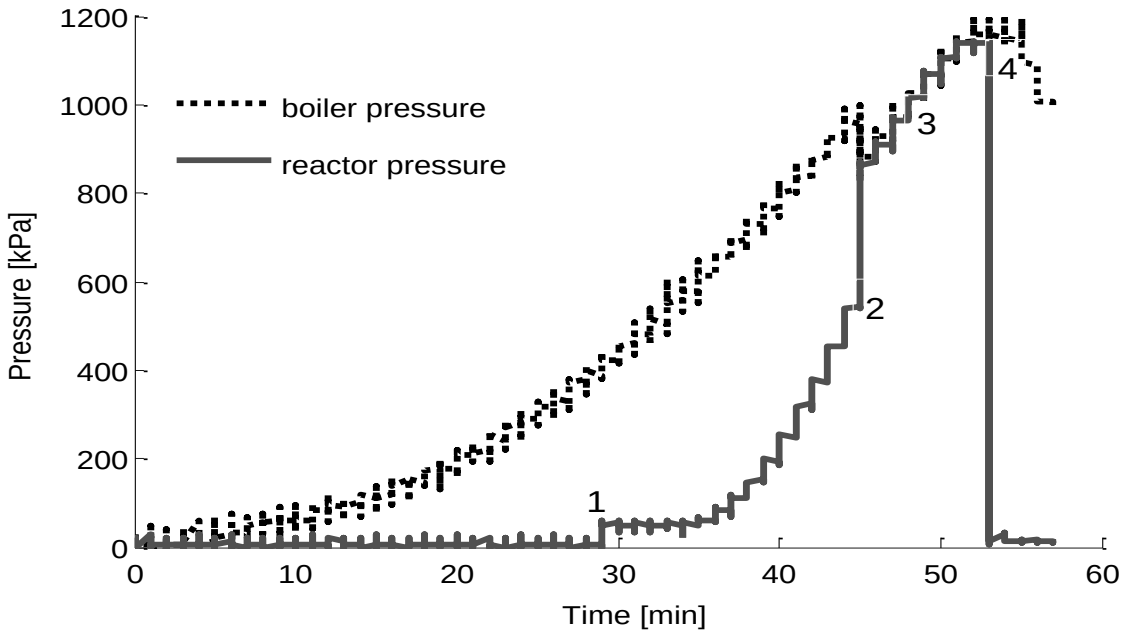


Figure 4.3 Typical pressure profile of an experiment (180^{o}C, 50% moisture content (w.b.) and 5 min retention time); Point 1 = reactor temperature was turn on; Point 2 = ball valve B-2 was opened; Point 3-4 = preset retention time; point 4 = ball valve B-1 was suddenly opened.

Figure 4.4 shows the steam exploded and non-treated biomass obtained from three different studies. This reflects that different biomass products can be obtained from steam explosion pretreatment depending on the nature and design of the steam explosion set up and the intended end use of the treated biomass products.

a)

b)

c)

Figure 4.4 - a. Comparison of non-treated and steam exploded barley straw grind at 180°C and 5 min retention time, with initial moisture content of 50% (w.b.) and ground using a hammer mill with 1.6 mm screen size (this present study). b. Comparison of non-treated and steam exploded (right) spruce grind at 220°C and 5 min retention time, with initial moisture content of 10% (w.b.) and ground using a hammer mill with 1.6 mm screen size, adapted from Tooyserkani et al. (2013). c. Comparison of non-treated and steam exploded (right) barley straw grind at 180°C and 4 min, with initial moisture content of 13.5% (w.b.) and ground using a hammer mill with 30 mm screen size, adapted from Adapa (2011).

4.4.1 Severity Factor

The severity factor used in this study combined temperatures (140-180^{o}C) and time (5-10 min) of the hydrolytic process in a single ordinate (R_o). Table 4.2 shows the severity of the pretreatment condition.

Table 4.2 Severity factor of the steam explosion process calculated based on equations 4.4.

Reaction temperature (^{o}C)	Residence time (min)	Severity factor
140	5	1.88
160	5	2.47
180	5	3.05
140	10	2.18
160	10	2.77
180	10	3.36

Number of replicates, n = 2

The severity factor obtained ranged from 1.88 to 3.36 depending on the temperature and residence time. It shows that the severity factor increased with temperature and time. Increasing temperature and time of steam explosion pretreatment will subsequently result in higher stringent pretreatment conditions of biomass. In the formulation of severity factor model, moisture content of biomass was not incorporated. The current investigation used moisture content of biomass to create a buffer effect and as a tool to dampen the severity of the pretreatment condition, and subsequently moderate the formation of inhibitors such as furfural. Therefore, the changes observed in the data suggested that the treatment severity (reaction ordinate) should also incorporate an additional function (moisture or acid or alkaline function) which will directly affect the hydrolytic mechanisms. This investigation shows that the reaction ordinate plays a vital role in the thermal degradation of cellulose and hemicellulose, in breaking the lignified matrix, changes in the ash content and color, elemental compositions, and inhibitor formation, as well as changes in the higher heating value of the pretreated biomass. Angles et al. (2003) applied the severity factor to the steam explosion of softwood with combined temperatures ranging from 176 to 231^{o}C and time (2.5-5.5 min) of the hydrolytic process in a single ordinate (R_o). These authors reported that the severity factor range was modified from log R_o = 2.6 to log R_o = 4.6. Chornet and Overend (1998) reported a severity factor of 2.6-4.2 during steam

explosion treatment of *Populus deltoides*. It was observed that the yield of sugars and degree of polymerization decreased with increasing severity factor.

Toussaint et al. (1991) and Heitz et al. (1991) demonstrated that the higher the operating conditions (temperature and time), the higher was the severity factor (R_o), resulting in a higher recovery of cellulose and lignin, which enhanced the accessibility and digestibility of cellulose. However, they stated that this recovery is at the expense of increasing the destruction and degradation of hemicellulose. It was suggested that the optimum treatment severity of each of the polymer fractions would probably involve a two-stage treatment cycle, with the removal of the bulk of the hemicellulose and lignin at low severity and a post-treatment to obtain the cellulose in high purity though at a low degree of polymerization (Heitz et al. 1991).

4.4.2 Chemical Composition of Steam Exploded and Non-Treated Lignocellulosic Biomass

4.4.2.1 Steam Treatment on Lignin

Table 4.3 shows that as the temperature increased, the AIL increased. This may be due to the carbonization of the sample resulting from the direct contact of biomass with the walls of the reactor. The analysis of variance performed on the data shows that temperature had a significant effect ($P<0.01$) on the AIL. At a higher temperature (180℃), exothermic reaction takes place, resulting in the degradation of hemicellulose and cellulose. Hemicellulose degrades easily and some volatile organic compounds vaporize as volatile components, while cellulose behaves as a fixed carbon (solid combustible residue). This may account for the increase in the acid insoluble lignin content. The percentage AIL increase was between 1.61 to 91.28% as compared to the non-treated biomass. This may also be attributed to lignin's high resistance to thermal degradation during the steam explosion process. Lam et al. (2011) investigated the steam explosion of Douglas Fir (*Pseudotsuga menziesii*) at a reaction temperature of 200-220^{o}C and a retention time of 5-10 min. These researchers reported that there was increase in total insoluble lignin content from 30 to 43% contributed by the thermal degradation of hemicellulose during the steam explosion treatment. Chen and Kuo (2011) reported that cellulose and lignin are both locked in biomass from the mild carbonization process. This indicates that the degraded cellulose may appear as residue resulting to the increase in the AIL.

Table 4.3 Soluble and insoluble lignin of steam exploded and non-treated barley straw

Temperature (^{o}C)	Moisture content (% mass fraction)	Retention time (min)	AIL (%)	ASL (%) Using UV-Vis Spec. (%)	Total acid soluble lignin (ng/mg)
NT	8	-	21.22 (0.36)	1.38 (0.03)	265.94 (8.87)
140	8	5	23.79 (1.44)	1.35 (0.11)	307.20 (4.88)
160	8	5	22.72 (0.43)	1.38 (0.02)	311.50 (10.26)
180	8	5	40.58 (2.44)	0.81 (0.04)	322.10 (11.35)
140	30	5	22.05 (0.17)	1.53 (0.04)	249.18 (20.52)
160	30	5	21.69 (0.80)	1.36 (0.06)	244.74 (6.19)
180	30	5	33.01 (1.20)	1.21 (0.06)	301.54 (12.20)
140	50	5	21.18 (0.10)	1.45 (0.01)	223.26 (11.39)
160	50	5	23.31 (0.08)	1.29 (0.10)	230.84 (32.51)
180	50	5	25.04 (0.66)	1.30 (0.04)	249.35 (15.83)
140	8	10	21.56 (0.61)	1.43 (0.08)	304.68 (1.73)
160	8	10	21.66 (0.55)	1.39 (0.05)	303.53 (6.22)
180	8	10	32.75 (0.36)	1.09 (0.07)	352.27 (11.10)
140	30	10	21.25 (0.45)	1.47 (0.04)	302.35 (4.65)
160	30	10	20.90 (0.20)	1.39 (0.04)	306.34 (15.71)
180	30	10	37.31 (1.88)	0.85 (0.04)	282.45 (27.76)
140	50	10	21.00 (0.26)	1.42 (0.05)	303.10 (10.08)
180	50	10	31.82 (0.40)	1.04 (0.05)	341.92 (5.40)

Value in parentheses = standard error; number of replicates, n = 2; NT = non-treated biomass; AIL = acid insoluble lignin; ASL = acid soluble lignin; Vis Spec. = visible spectroscopy; all % values are in mass fraction.

At the investigated temperatures in this current study the steam exploded biomass gave off moisture, began to turn brown, lost its hygroscopic properties and became more friable than non-treated biomass. Similar results have been reported by Excoffier et al. (1998) and Ferro et al. (2004) on lignocellulosic biomass. This current study shows that AIL increased as the moisture content decreased from 50 to 8% (mass fraction of water) Statistical analysis showed that moisture content of biomass had a significant effect ($P<0.01$) on the AIL. This demonstrate that biomass initial moisture content has buffer effect by damping the severity of the pretreatment condition; thereby, avoiding the degradation of the amorphous portion of the structural carbohydrates that could be deposited as residue and subsequently increasing the quantity of AIL in the treated biomass. The interaction between temperature and moisture content and also between moisture content and retention time had a statistically significant effect ($P<0.01$) on the

AIL. The interaction among the three variables (temperature, moisture content, and retention time) also had a significant effect on the AIL. It can be observed from Table 4.3 that the higher the temperature and retention time, the higher the total acid soluble lignin moieties content. At higher temperature and retention times the biomass matrix was deconstructed, which enhanced the assessment of the total acid soluble lignin moieties. Cullis et al. (2004) reported that as the relative severity increased, the extent of lignin modification also increased. This current investigation depicts that the optimum pretreatment condition (180^{o}C, 8% (w.b.), and 10 min retention time) resulted in the maximal effect of pretreatment, with yields of the total acid soluble lignin moieties 33% greater than from the non-treated biomass. Easy access to the total acid soluble lignin moieties is associated with the breakdown of the lignified biomass matrix. This reflects maximum enhancement of the accessibility and digestibility of the cellulose and hemicellulose by 33% during the enzymatic reaction. This will also help in decreasing the costs and amount of enzymes required for the next stage of enzymatic hydrolysis by about 33% compared to the non-treated biomass. The higher the moisture content, the lower is the total acid soluble lignin moieties, because of the buffer effect of moisture and it requires higher energy to evaporate the moisture before deconstructing the biomass matrix. Cullis et al. (2004) studied the effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. It was reported that moisture content plays an influential role in the severity of pretreatment, as it greatly influences the ability of heat and chemicals to penetrate wood. In this present investigation, analysis of variance performed on the data shows that temperature, retention time, and moisture content had a significant effect ($P<0.05$, $P<0.01$, and $P<0.01$, respectively) on the total soluble lignin moieties. The interaction between moisture content and retention time, and the interaction among moisture content, retention time, and temperature had a significant effect ($P<0.05$) on the soluble lignin moieties.

4.4.2.2 Steam Treatment on Inorganics and Furfurals

The ash content in the steam explosion pretreated ground barley straw increased as the temperature and retention time increased (Table 4.4). Both variables had a significant effect ($P<0.01$) on the ash content of the steam exploded biomass. This could also be due to the degradation of the amorphous portion of the hemicellulose and cellulose. High ash content is not

a desirable quality of biomass feedstock for thermochemical conversion; however, the percentage increase (5.68-28.10%) in ash content of steam explosion pretreated barley grind is not so high as to drastically affect biomass combustion. In comparison with the non-treated biomass, it is evident that the three independent variables studied have a strong contribution to the dependent variables (ash content and AIL).

Table 4.4 Furfurals and ash content of steam exploded and non-treated barley straw

Temperature (^{o}C)	Moisture content (% mass fraction)	Retention time (min)	Average furfural (%)	Ash content (%)	Increase/ decrease in ash (%)
NT	8	-	4.04 (0.11)	5.42 (0.06)	0.00
140	8	5	5.17 (2.97)	5.75 (0.09)	5.95
160	8	5	5.70 (2.17)	5.74 (0.05)	5.86
180	8	5	6.29 (0.33)	6.93 (0.16)	27.82
140	30	5	3.99 (0.20)	5.86 (0.01)	8.09
160	30	5	5.13 (0.35)	5.40 (0.01)	-0.34
180	30	5	5.86 (0.14)	6.39 (0.17)	17.90
140	50	5	4.51 (2.74)	5.75 (0.26)	6.05
160	50	5	3.82 (1.40)	5.82 (0.08)	7.24
180	50	5	5.70 (0.19)	6.16 (0.15)	13.61
140	8	10	5.99 (2.87)	5.92 (0.00)	9.21
160	8	10	5.98 (0.44)	6.01 (0.26)	10.77
180	8	10	7.95 (0.24)	6.79 (0.21)	25.22
140	30	10	4.98 (0.55)	5.73 (0.14)	5.68
160	30	10	4.70 (1.13)	6.22 (0.48)	14.76
180	30	10	5.74 (0.54)	6.95 (0.06)	28.10
140	50	10	4.15 (2.48)	5.80 (0.15)	6.99
180	50	10	5.87 (0.28)	6.81 (0.34)	25.48

Value in parentheses = standard error; number of replicates, n = 2; NT = non-treated biomass; all % values are in mass fraction.

The formation of furfural was also dependent on the severity of the pretreatment conditions. The higher the temperature and retention time, the higher was the furfural content (Table 4.4). This is due to the reactions and degradations of xylose and glucose from hemicellulose and cellulose, respectively, that took place at severe pretreatment conditions. Figure 4.5 shows the effects of severity factor on the formation of furfural in the biomass as the biomass pretreatment conditions changed.

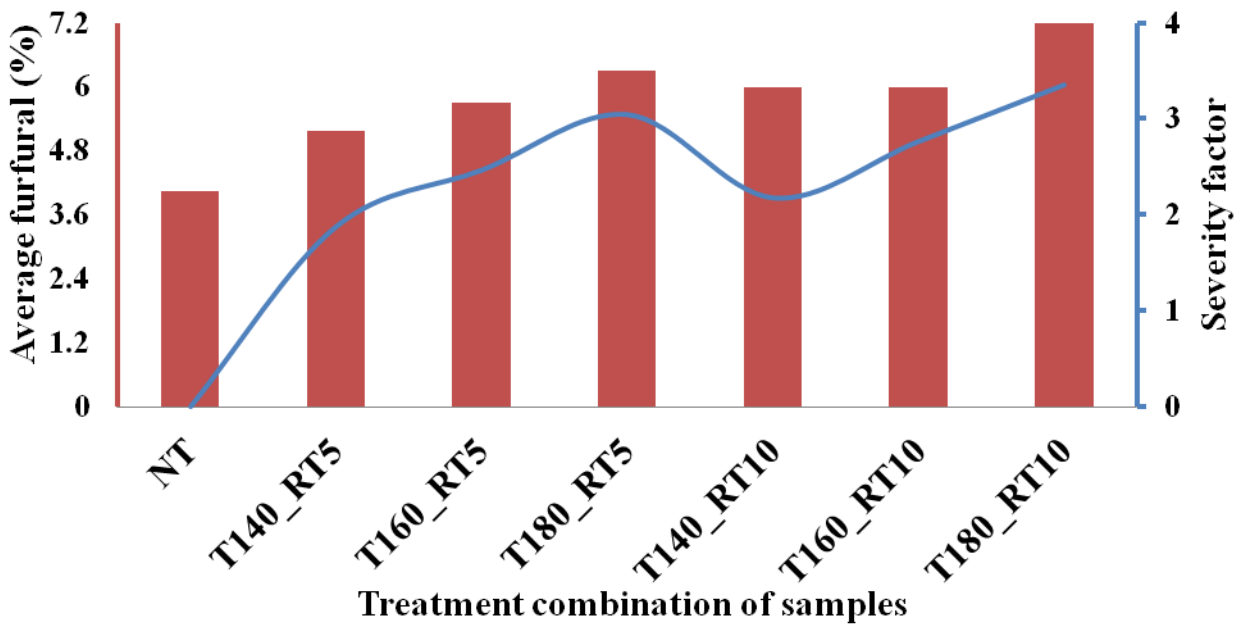


Figure 4.5. Effects of biomass pretreatment conditions on the formation of furfural. NT = non-treated biomass; T140_RT5 = pretreatment temperature, T @ 140^{o}C and retention time, RT @ 5 min etc.

Moisture content varied inversely with furfural content. The higher the moisture content, the lower the furfural content, because the system was dampened and subsequently reduced the harsh pretreatment environment that would have led to formation of high degree of inhibitors. Analysis of variance showed that moisture content significantly affected ($P<0.05$) the formation of furfural. Toussaint et al. (1991), Tanahashi et al. (1988), Chornet and Overend (1998), and Kaar et al. (1991) reported that at excessive conditions (high temperatures and pressures) during steam explosion, degradation of xylose to furfural and glucose to 5-hydroxymethyl furfural occurred. Furfural is an undesirable compound in fermentation because it inhibits microbial growth and activity (Chornet and Overend 1998). Chornet and Overend (1998) and Wang et al. (2009) reported that the retention time and temperature are the process parameters required for the optimization of steam explosion process. However, these researchers demonstrated that long retention time increases the production of inhibitory substances, and has to be minimized to avoid this.

4.4.2.3 Steam Treatment on Cellulose and Hemicellulose

Table 4.5 Cellulose and hemicellulose content of steam exploded and non-treated barley straw

Temperature (^{o}C)	Moisture content (% mass fraction)	Retention time (min)	Cellulose (%)	Cellulose loss (%)	Hemicellulose (%)	Hemicellulose loss (%)
NT	8	-	38.83(9.10)	-	31.68(8.86)	-
140	8	5	11.19(0.36)	71.20	3.42(0.05)	89.21
160	8	5	11.17(1.14)	71.23	3.29(0.06)	89.61
180	8	5	9.58(0.01)	75.32	3.74(0.63)	88.19
140	30	5	14.82(0.17)	61.83	3.80(0.07)	88.01
160	30	5	14.63(1.36)	62.34	4.13(0.05)	87.75
180	30	5	12.07(0.40)	68.91	3.88(0.48	81.11
140	50	5	15.81(0.90)	59.28	6.58(0.36)	79.22
160	50	5	14.51(1.07)	62.63	5.11(0.82)	83.88
180	50	5	15.77(0.16)	59.38	5.23(0.55)	83.49
140	8	10	10.02(0.34)	74.20	3.90(0.35)	87.69
160	8	10	9.11(0.47)	76.53	4.47(1.38)	85.89
180	8	10	8.74(1.15)	77.49	3.23(0.06)	89.81
140	30	10	15.38(1.11)	60.39	5.03(0.56)	84.12
160	30	10	13.57(0.43)	65.07	6.59(0.13)	79.20
180	30	10	12.70(1.29)	67.31	4.54(0.05)	85.67
140	50	10	16.02(0.06)	58.76	6.33(0.67)	80.02
180	50	10	12.35(2.13)	68.21	4.69(0.58)	85.20

Value in parentheses = standard error; number of replicates, n = 2; NT = non-treated biomass; all % values are in mass fraction.

Temperature played an important role in steam explosion process (Table 4.5). In comparison with the non-treated biomass, it is evident that temperature, moisture content, and retention time had a significant effect on the cellulose and hemicellulose content. The degradation of simple sugars increased at higher temperature and retention time. Also, higher moisture content resulted in less degradation of the simple sugars. High feedstock moisture content acts as acid catalyst to hydrolyze biomass during steam explosion. However, the direct contact of biomass with the walls of the reactor will limit and affect the extent of the hydrolysis. Therefore, a combination of carbonization and acid catalyzed hydrolysis occurred which ultimately led to the degradation of the simple sugars and increase in the AIL. These results indicated that the hemicellulose contained in the biomass was highly degraded compared to cellulose. Cellulose loss (degradation) was between 58 and 77%, while hemicellulose degradation was between 79 and

89%. The high degradation of hemicellulose was due to its amorphous nature, which degrades easily and evaporates as volatile components during the carbonization process. Presumably, the crystallinity of cellulose was responsible for the less degradation. These degradations can be explained by considering the monomers of hemicellulose and cellulose which consist primarily of sugars. Statistical analysis shows that temperature and moisture content had a significant effect ($P<0.01$) on the structural and chemical compositional changes of cellulose. Temperature also significantly affected ($P<0.01$) the changes associated with hemicellulose. Degradation of cellulose and hemicellulose during steam explosion/thermal pretreatment of biomass has been reported by Toussaint et al. (1991), Excoffier et al. (1998), Ferro et al. (2004), Yan et al. (2009), and Chen and Kuo (2010). It was reported that hemicellulose is very reactive and was nearly completely removed at 200^{o}C, while both cellulose and lignin can be dissolved partially at higher temperatures. Shaw (2008) performed steam explosion on poplar and wheat straw at 200-205^{o}C, steam pressure of 1.66-1.73 MPa for 4-4.5 min. This author reported a decrease in cellulose and hemicellulose content with an increase in the lignin content after the steam explosion treatment of both biomass samples.

Chen and Kuo (2011), Yang et al. (2007), and Khezami et al. (2005) studied the effects of thermal process on biomass. These researchers showed that thermal pretreatment removes moisture and light volatiles from biomass. Bergman et al. (2005) and Lipinsky et al. (2002) reported that during the torrefaction process, biomass was partly decomposed giving off various condensable and non-condensable gases, with a carbon rich solid as a final product. Mohammad and Karimi (2008) reported a corresponding decrease in total sugar recovery with increasing temperature during steam explosion.

4.4.2.4 Ultimate Analysis of Steam Exploded and Non-Treated Barley Straw Grind

Table 4.6 shows that the elemental composition of biomass was dependent on the severity of the treatment condition. Increased carbon content of biomass resulted from higher severity of steam explosion pretreatment. The initial carbon and oxygen content of the non-treated barley straw was 42.30% and 50.99%, respectively. After the steam pretreatment (at 160-180^{o}C, 8% mass

fraction of water, and 5 min retention time), the carbon content increased to a maximum of 46.69-46.89%, while the oxygen content decreased to 45.76-46.77%.

Table 4.6 Elemental composition of steam exploded and non-treated barley straw grind

Temp. (^{o}C)	M.C. (%)	RT (min)	N (%)	C (%)	S (%)	H (%)	O* (%)
NT	8	-	0.31 (0.01)	42.30 (0.04)	0.17 (0.08)	6.23 (0.15)	50.99(0.08)
140	8	5	0.37 (0.02)	42.49 (0.01)	0.07 (0.01)	6.37 (0.03)	50.70(0.00)
160	8	5	0.35 (0.02)	46.89 (2.84)	0.05 (0.00)	6.96 (0.44)	45.76(3.29)
180	8	5	0.41 (0.00)	46.69 (0.04)	0.05 (0.01)	6.08 (0.02)	46.77(0.06)
140	30	5	0.34 (0.01)	42.17 (0.06)	0.04 (0.01)	6.38 (0.01)	51.07(0.07)
160	30	5	0.34 (0.01)	42.67 (0.03)	0.09 (0.03)	6.31 (0.03)	50.60(0.05)
180	30	5	0.38 (0.00)	45.19 (0.04)	0.04 (0.01)	6.11 (0.03)	48.28(0.08)
140	50	5	0.37 (0.01)	42.10 (0.02)	0.04 (0.01)	6.41 (0.02)	51.07(0.04)
160	50	5	0.37 (0.00)	43.25 (0.17)	0.26 (0.05)	6.58 (0.05)	49.54(0.27)
180	50	5	0.39 (0.00)	43.65 (0.03)	0.12 (0.02)	6.30 (0.01)	49.55(0.05)
140	8	10	0.35 (0.01)	43.04 (0.07)	0.07 (0.01)	6.33 (0.05)	50.21(0.13)
160	8	10	0.38 (0.01)	42.83 (0.26)	0.08 (0.01)	6.19 (0.09)	50.53(0.36)
180	8	10	0.43 (0.01)	46.66 (0.07)	0.08 (0.01)	6.15 (0.01)	46.67(0.07)
140	30	10	0.36 (0.01)	42.39 (0.04)	0.05 (0.01)	6.29 (0.02)	50.91(0.04)
160	30	10	0.39 (0.01)	41.93 (0.20)	0.12 (0.05)	6.22 (0.03)	51.34(0.28)
180	30	10	0.44 (0.00)	46.63 (0.21)	0.07 (0.00)	5.91 (0.06)	46.95(0.28)
140	50	10	0.38 (0.01)	42.01 (0.03)	0.09 (0.00)	6.35 (0.04)	51.17(0.06)
180	50	10	0.46 (0.01)	45.54 (0.30)	0.07 (0.01)	6.11 (0.05)	47.83(0.34)

Value in parentheses = standard error; number of replicates, n = 2; Temp. = temperature; M.C. = moisture content (% mass fraction); RT= retention time; min = minute; N = Nitrogen; C = Carbon; S =Sulfur; and H = Hydrogen; NT = non-treated; std = standard; O* = oxygen obtained by difference; NT = non-treated biomass; all % values are in mass fraction.

The carbon content of the steam exploded solid product significantly increased ($P<0.01$) as a function of temperature and retention time, while hydrogen and oxygen content decreased. The carbon content increased from 43.65% to 45.54% as the retention time increased from 5 to 10 min (at 180^{o}C and 50% mass fraction of water). However this was not the case for all the collected data. Similar results have been obtained from the ultimate analysis performed on thermal pretreated biomass feedstock by Ferro et al. (2004) and Yan et al. (2009). Angles et al. (2003) reported that the carbon content of the steam exploded pretreated samples increases because lignin condenses and even carbonizes. This condensation may be accompanied by water

loss as evidenced by a decrease in oxygen content within the pretreated lignin. Loss in hydrogen and oxygen may be due to the formation of water and carbon monoxide and dioxide.

The reactions that occur during steam explosion generally cause smaller hydrocarbon molecules (volatiles and gases, not recorded in this current study) with low energy density to volatilize or escape, which in turn increase the energy density of the remaining carbon-rich solids. The results obtained from this current elemental ultimate analysis is consistent with the results shown in Tables 4.3-4.5, indicating that at higher temperature and retention times, there was carbonization (increase in carbon content), degradation of the sugars (cellulose and hemicellulose), breakdown of the biomass matrix (cross-linking lignin), all of which subsequently led to higher yield of the total soluble lignin moieties during the two step acid hydrolysis. The nitrogen content also increased with increasing process temperature and retention time.

As the moisture content of barley straw grind increased from 8 to 50% (mass fraction of water) at 180^{o}C and 5 min retention time, the carbon content decreased accordingly from 46.69% to 43.65%, while the hydrogen and oxygen contents increased from 6.08% and 46.77% to 6.30% and 49.55%, respectively. The change in carbon, hydrogen, and oxygen content were consistent and significant, but less dramatic at high moisture content. This implies that higher energy is required to evaporate the moisture before carbonization of the sample starts. The statistical analysis shows that moisture content significantly affected ($P<0.01$) the carbon content of the steam exploded biomass.

4.4.2.5 Higher Heating Value of Steam Exploded and Non-Treated Biomass

From Table 4.7, the H/C and O/C ratios were relatively constant with temperature, moisture content, and retention time. The measurement of HHV was initiated based on the consideration that the SE pretreated biomass could as well be used for the production of electricity or home heating during the winter season. The second reason of measuring the HHV was to confirm the carbonization that took place during the SE pretreatment. Because carbon content of biomass has a direct correlation with HHV. There is a close relationship between the theoretical HHV (17.31 MJ kg^{-1}) of barley straw (reported by Annamalai et al. (1987) and the experimentally obtained HHV (17.47 MJ kg^{-1}) of the non-treated barley straw (from this current investigation). The HHV

of the solid product increased with temperature and decreased with increasing moisture content. The HHV increased up to a maximum of about 11% as compared with the non-treated biomass. This is a reflection of the increased carbon content with decreased in oxygen content as the temperature increased. ANOVA showed that temperature and moisture content significantly affected ($P<0.01$ and $P<0.05$, respectively) the HHV.

Table 4.7 Ratio of elemental composition, degree of carbonization, higher heating value, mass yield, and energy recovery of steam exploded pretreated and non-treated barley straw grind

Tempt. (^oC)	M.C. (%)	RT (min)	H/C	O/C	%DOC	HHV (MJ kg^{-1})	MY (%)	ER (%)
NT	8	-	0.15	1.21	-	17.47	-	-
140	8	5	0.15	1.19	1.00	17.51	95.14	95.35
160	8	5	0.15	0.98	1.00	17.52	77.30	77.51
180	8	5	0.13	1.00	1.11	19.36	82.55	91.45
140	30	5	0.15	1.21	1.00	17.46	85.51	85.47
160	30	5	0.15	1.19	1.00	17.46	69.90	69.84
180	30	5	0.14	1.07	1.06	18.61	74.25	79.07
140	50	5	0.15	1.21	1.01	17.57	84.75	85.21
160	50	5	0.15	1.15	1.02	17.75	85.26	86.62
180	50	5	0.14	1.14	1.03	18.05	80.76	83.42
140	8	10	0.15	1.04	1.00	17.48	93.90	93.92
160	8	10	0.14	1.18	1.01	17.67	87.99	89.00
180	8	10	0.13	1.00	1.09	19.00	73.17	79.56
140	30	10	0.15	1.20	1.00	17.52	81.61	81.83
160	30	10	0.15	1.22	1.00	17.52	96.14	96.37
180	30	10	0.15	1.22	1.09	19.02	78.45	85.39
140	50	10	0.15	1.22	1.00	17.48	76.53	76.56
180	50	10	0.13	1.05	1.07	18.64	70.81	75.53

Tempt. = temperature; M.C. = moisture content (% mass fraction); RT= retention time; min = minute; C = Carbon; H = Hydrogen; O = oxygen; DOC = degree of carbonization; HHV = higher heating value; MY = mass yield; ER = energy recovery; NT = non-treated biomass; n = 2.

Lam et al. (2011) and Kumar et al. (2010) reported an increase in HHV, lignin, and ash content after steam pretreatment of whitewood samples. Yan et al. (2009) reported increased HHV with temperature resulting from thermal pretreatment of loblolly pine. Adapa (2011) determined the HHV of the non-treated and steam exploded (at 180^oC, steam pressure of 900 kPa for 4 min) barley straw and found the HHV of non-treated and steam exploded barley straw to be 16.40 MJ

kg^{-1} and 17.40 MJ kg^{-1}, respectively. Cellulose, hemicellulose, and lignin are the major components of lignocellulosic biomass. Therefore, any chemical, physical, or structural changes in their composition could potentially lead to changes in the HHV of the biomass.

This investigation shows that reaction temperature is a significant variable in pretreatment processes; increasing temperature caused a reduction in both mass yield and energy recovery. However, this trend was not consistent in all cases. Statistical analysis showed that temperature significantly affected ($P<0.01$) the mass yield and energy recovery of the steam exploded biomass. This mass loss may be associated with the decomposition/degradation of some reactive components of the hemicellulose (such as acetic acid), as well as moisture loss. At high residence times, the mass loss can be attributed to the decomposition of the less reactive components of the hemicellulose. This two-step nature of the carbonization process (decomposition of reactive and less reactive components of biomass) has been reported by Arias et al. (2008), who investigated the influence of carbonization on the grindability and reactivity of woody biomass. Yan et al. (2009) reported a similar trend in the thermal pretreatment of loblolly pine, where there was a decrease in the mass and energy yield of the pretreated biomass as the reaction temperature increased. This may be associated to the loss of some volatiles and gases during the thermal pretreatment process. Over the moisture content ranges studied, wet biomass resulted in a lower mass yield and energy recovery as compared to drier biomass with higher mass yield and energy recovery.

4.4.2.6 Degree of Carbonization (DOC)

The DOC increased with temperature and slightly decreased with moisture content of the biomass. This again shows that the water present in the biomass can acts as a damper to reduce the severity of the pretreatment conditions. Analysis of variance showed that temperature played a significant role (with $P<0.01$) on the DOC of the steam exploded biomass. Tooyserkani et al. (2013) steam pretreated wood species (10% mass fraction of water) at 220^{o}C for 5 min to determine the DOC (torrefaction). The authors reported that the calorific value increased as a result of steam explosion with the highest increase of 26% for spruce, from 18.7 MJ kg^{-1} to 23.6 MJ kg^{-1}. It was also reported that there was a significant increase in the ash content. The ash

content almost doubled from 2.11% to 4.13% after steam explosion. In this study, we observed the following as indicators of carbonization: color, HHV, carbon content, lignin content, sugars, and ash content.

4.4.2.7 Color Analysis

Table 3.8 shows the mean values of the color parameters of L*, a*, and b* of the steam exploded and non-treated samples. Color characteristics of the steam explosion pretreated samples are a reflection of the severity of the pretreatment conditions. The lightness (L*) of steam exploded ground barley straw decreased by up to a maximum of about 51% with increasing temperature and retention time. This implies that, the higher the severity of the pretreatment conditions, the darker were the pretreated samples. In most cases, the yellowness of the steam exploded samples decreased with increasing temperature and retention time, except for samples pretreated at 160^{o}C, moisture content of 8-30% (mass fraction of water) and at 5-10 min retention time. Increasing blueness also signifies that the samples get darker as the severity of pretreatment increased with respect to increasing temperature and retention time.

Table 4.8 Color characteristics of steam exploded and non-treated ground barley straw

Temp. (^oC)	Moisture content (% mass fraction)	Retention time (min)	L*	a*	b*	ΔE*
NT	8	-	60.83 (0.01)	7.67 (0.01)	20.36 (0.02)	-
140	8	5	55.82 (0.01)	8.31 (0.01)	16.97 (0.01)	6.08 (0.01)
160	8	5	54.68 (0.00)	9.13 (0.01)	18.24 (0.01)	6.67 (0.02)
180	8	5	29.90 (0.02)	7.23 (0.03)	8.24 (0.02)	33.22 (0.02)
140	30	5	57.10 (0.01)	8.74 (0.00)	20.29 (0.02)	3.88 (0.01)
160	30	5	47.60 (0.00)	9.03 (0.01)	20.11 (0.01)	13.30 (0.01)
180	30	5	32.93 (0.02)	9.08 (0.00)	13.35 (0.02)	28.80 (0.00)
140	50	5	50.37 (0.00)	9.01 (0.00)	19.16 (0.01)	10.61 (0.01)
160	50	5	41.78 (0.00)	8.28 (0.01)	15.65 (0.01)	19.63 (0.01)
180	50	5	31.59 (0.01)	6.75 (0.01)	7.40 (0.02)	32.00 (0.02)
140	8	10	53.15 (0.01)	9.20 (0.00)	19.34 (0.00)	7.89 (0.01)
160	8	10	45.90 (0.01)	10.18 (0.01)	17.24 (0.01)	15.46 (0.01)
180	8	10	30.98 (0.00)	9.18 (0.01)	12.45 (0.02)	30.91 (0.01)
140	30	10	51.07 (0.00)	9.23 (0.01)	17.68 (0.01)	10.24 (0.01)
160	30	10	50.99 (0.01)	9.58 (0.00)	19.16 (0.01)	10.09 (0.01)
180	30	10	30.44 (0.01)	8.01 (0.01)	8.53 (0.01)	32.62 (0.01)
140	50	10	47.65 (0.01)	9.25 (0.00)	17.94 (0.00)	13.49 (0.01)
180	50	10	30.41 (0.01)	6.58 (0.01)	7.14 (0.00)	33.18 (0.01)

Value in parentheses = standard error; number of replicates, n = 3; Temp. = temperature; The scale L* represents from 0 for black to +100 for white; The a* and b* have no specific numerical limits; Positive a* is red; Negative a* is green; Positive b* is yellow; Negative b* is blue; and ΔE* = total color difference.

The lightness (L*) and yellowness (b*) of the non-treated ground barley straw were higher as compared to the steam pretreated samples as it is evident in Figure 4.4a. The calculated differential color composite ΔE* is a good indicator of color deviation between pretreated and non-treated samples. The total color difference between the steam exploded and non-treated samples increased with increasing temperature and retention time. The highest total color difference was observed at 180^oC. Further studies may be required to rapidly estimate and correlate color and HHV of steam exploded biomass at various severity factors. The color of steam pretreated samples can be used as an indicator of carbonization during steam explosion pretreatment, as samples tend to darken with increased carbonization (Tooyserkani et al. 2013). Zhang and Cai (2006) reported that the color changes observed in wood during steam treatment were as a result of a series of chemical reactions. These are primarily due to the reactions

between the chemical constituents of wood cell wall and extractives under high temperature and humidity conditions. Cellulose and hemicellulose are polysaccharides that can cause significant changes at a critical temperature of 120^{o}C. Cellulose degrades to yellow when the temperature is above 140^{o}C (Zhang and Cai 2006). From the collected data in this study, hemicellulose is more sensitive to temperature and easier to degrade than cellulose. During steam explosion pretreatment, furfural and some polysaccharides with low molecular weights were generated from the degradation of hemicellulose (Zhang and Cai 2006). Changes in lignin and extractives can also contribute to the color changes. Carboxylates and phenol may be produced within the lignin or extractive molecules at high temperatures and humidity (Zhang and Cai 2006). All these put together may lead to the current color changes in the ground barley straw during steam explosion pretreatment.

4.5 Conclusion

The operating reaction temperature, retention time, and moisture content of biomass barley straw grind were studied to investigate the dynamics responsible for the changes in the steam exploded barley straw. The severity factor, chemical compositions, ultimate elements, higher heating value, and color characterization of pretreated barley straw were used as indicators to evaluate the effects of the main factors. The results obtained from these investigations provided qualitative and quantitative insights into the responses of hemicellulose, cellulose, and lignin in barley straw to the steam explosion process at various levels of the variables tested. Higher temperature and retention time resulted to the breakdown of the lignified barley straw matrix with increased in the total acid soluble lignin by up to a maximum of about 33%. The AIL increased with increasing temperature and decreasing moisture content by up to a maximum of about 91.28% as compared to the non-treated biomass. Both HHV and carbon content increased by up to a maximum of about 11%, while the severity factor increased correspondingly with increasing temperature and retention time. The lightness (L) of steam exploded ground barley straw decreased by up to a maximum of about 51% with increasing temperature and retention time. Therefore, it is clear that the thermal degradation of cellulose and hemicellulose in barley straw are different from each other due to the crystalline nature of the former and the amorphous nature of the latter compound. The high initial moisture content of ground barley straw has

buffer effect by lessening the effect of harsher pretreatment conditions. Based on the collected data, barley straw can be used as a potential feedstock for the biofuel industry.

Chapter 5

5. Enzymatic Saccharification of Radio Frequency-Alkaline and Steam Exploded Pretreated and Non-Treated Lignocellulosic Barley Straw Grind

A portion of a similar version of this chapter has been presented at the CSBE/SCGAB Annual General Meeting and Technical Conference:

- Iroba, K.L., L.G. Tabil, T. Dumonceaux and S. Sokhansanj. 2013. Enzymatic saccharification of radio frequency alkaline pretreated and non-treated lignocellulosic biomass barley straw. Paper No. CSBE13-016. Conference for Interdisciplinary Engineering and CSBE/SCGAB AGM, University of Saskatchewan, Saskatoon.

Contributions of Ph.D. Candidate

The present study involves the development of a protocol that could be used to evaluate or assess the effects of different pretreatment approaches by enzymatically hydrolyzing pretreated lignocellulosic biomass. The biofuel industry can subsequently use this protocol for the quantification of the glucose present in the hydrolyzed samples. All the experiments, data analysis and manuscript writing were performed by Kingsley Iroba with technical assistance of Dr. Dumonceaux. Dr. Tabil provided editorial input.

Contributions of this Paper to Overall Study

Knowledge Gap: In literature, enzymatic hydrolysis of various pretreated lignocellulosic biomass has been reported. However, no studies were found on the enzymatic hydrolysis of radio frequency pretreated barley straw grind at various levels of ratio of biomass to alkaline (NaOH) solution. Also, no investigation has been reported on steam explosion pretreatment of barley straw grind at low severity factor. Therefore, the objective of this present study was to validate and evaluate the readily available enzyme digestible glucose (cellulose) in the radio frequency alkaline and steam explosion pretreated and non-treated lignocellulosic barley straw grind.

Justification: For the production of bioethanol from lignocellulosic biomass (fermentation process), the lignified bonds in biomass need to be efficiently broken and disrupted via pretreatment, and also enzymatically hydrolyzed. This is due to the recalcitrance and resistance of lignocellulosic biomass to enzymatic attack, as such they must be pretreated to alter the structure and compositions of the substrate, and partially remove lignin before it can be effectively and enzymatically hydrolyzed (Chandra et al. 2007; Mohammad and Karimi 2008).
The structurally bonded chemical components of the native lignocellulosic biomass have significant effect on the required amount of enzymes and yeasts during hydrolysis and fermentation process, respectively. Li et al. (2004) and Mais et al. (2002) reported that pretreatment of biomass (ball milling) improves the rate of saccharification/hydrolysis and reduce the amount of enzyme required to attain total hydrolysis of the carbohydrate moieties.

5.1 Abstract

Lignocellulosic biomass in the form of crop straw, an abundant agricultural residue, can be utilized as feedstock for bioethanol production. However, the complex lignocellulosic nature of biomass makes the process uneconomically feasible. This drawback necessitates the breakdown and disruption of the lignified matrix to enhance easy and economical access to the energy potentials of cellulose and hemicellulose. Barley straw was subjected to radio frequency alkaline and steam explosion pretreatment to increase the degradability, accessibility, and digestibility of the energy potentials. The effect of the pretreatment was assessed through chemical composition analysis using the National Renewable Energy Laboratory (NREL) standard method. The radio

frequency alkaline and steam explosion treatment combinations (ratio 1:6 of biomass to NaOH solution at 24^{o}C-90^{o}C for radio frequency; while for steam explosion: 8-50% (w.b.) biomass moisture content, 5-10 min retention time and at 140-180^{o}C) that resulted to optimum yield of cellulose and hemicellulose were selected and then enzymatically digested with a combined mixture of cellulase and β-glucosidase enzymes at 50^{o}C for 96 h on a shaking incubator at 250 rev/min. The glucose in the hydrolyzed samples was subsequently quantified. The results obtained confirmed the effectiveness of the pretreatment processes. The average available percentage glucose yield that was released during the enzymatic hydrolysis for bioethanol production ranged from 78 to 96% for radio frequency alkaline pretreated samples and 30 to 50% for the steam explosion pretreated samples, depending on the treatment combination. While the non-treated sample has available average percentage glucose yields of just below 12%.

5.2 Introduction

Lignocellulosic agricultural and forest biomass residues have been identified as potential feedstock for industrial bioethanol production. Cellulose and hemicellulose are the major components of biomass. These are largely protected from attack by enzymes (Söderström et al. 2003; Fan et al. 2006a). This inaccessibility to enzymatic attack is mainly due to the association of these polysaccharides with lignin and with each other, all of these collaboratively act as great barrier shielding the polysaccharides from hydrolysis (Chandra et al. 2007; Mohammad and Karimi 2008). Due to the recalcitrance and resistance to enzymatic attack, the lignocellulosic biomass must be pretreated to alter the structure and compositions of the substrate, and partially remove lignin before it can be effectively and enzymatically hydrolyzed (Chandra et al. 2007; Mohammad and Karimi 2008). A suitable pretreatment should result in reduction of the lignin content, an increase in surface area and porosity of the biomass particles, eliminate the natural resistance of cellulose towards cellulase activity, increase the accessibility and digestibility of cellulose and hemicellulose, limit the formation of inhibitors (such as furfural, 5-hydroxyl-methyl furfural, formic acid, vanillic acid, syringic acid and syringylaldehyde), and should be cost effective (Qi et al. 2009; Jørgensen et al. 2007; Li et al. 2004; Jacobus 1997). Different approaches, such as mechanical, chemical, thermochemical, and biological pretreatment, have been applied to lignocellulosic biomass (Sun and Cheng 2002). Li et al. (2004) and Mais et al.

(2002) reported that pretreatment such as ball milling, significantly increased yield and selectivity of enzymatic saccharification, due to the enhancement of the accessible surface area and the average size of the capillaries within the biomass particles. These are two of the major factors limiting efficient hydrolysis of biomass. Iroba et al. (2013) investigated the application of radio frequency (RF) alkaline pretreatment on lignocellulosic barley straw. It was reported that RF-alkaline pretreatment of barley straw resulted in lower acid insoluble lignin and higher total acid soluble lignin moieties. Li et al. (2004) and Mais et al. (2002) reported that pretreatment of biomass by ball milling improved the rate of saccharification and reduced the amount of enzyme required to attain total hydrolysis of the carbohydrate moieties.

Insoluble cellulose chains can be disrupted or broken down to form soluble glucan chains and sugars such as single glucose sugar molecules by enzymes known as cellulase (Kumar et al. 2009; Jacobus 1997). Cellulase could be refers to a group of enzymes produced chiefly by bacteria, fungi, and protozoans that catalyze the hydrolysis of cellulose (Kumar et al. 2009). However, cellulases can also be produced by animals and plants. Such reaction happens in the stomachs of ruminants (at body temperature) such as cows and sheep, where the enzymes are produced by intestinal bacteria (Kumar et al. 2009). Lignocellulosic biomass materials can correspondingly be enzymatically hydrolyzed under comparatively light conditions (50°C and pH ~ 5), enabling efficient cellulose disruption/breakdown without the formation of inhibitors or byproducts that may prevent or inhibit optimal enzyme activity (Kumar et al. 2009). Enzymes work synergistically to hydrolyze cellulose by generating new accessible sites for each other, hence, eliminating obstacles, and mitigating the formation of product inhibition (Jørgensen et al. 2007; Eriksson et al. 2002). Wen et al. (2004) reported that the enzymatic process is considered to be the most favorable and promising technology for bioconversion of lignocellulosic biomass to monomeric sugars, because enzymatic hydrolysis of biomass is milder, more specific, and does not lead to the formation of byproducts unlike the dilute acid conversion process. However, the current bioconversion process is not economically feasible, due to the fact that enzymatic hydrolysis is slow and requires more enzyme loading to effectively realize reasonable conversion rates and yields, which are due to the influence of the substrate factors discussed above, as well

as some mechanistic factors such as cellulase adsorption and synergism (Lynd et al. 2002; Mansfield et al. 1999; Wyman 1999).

Cellulose-based enzymatic hydrolysis (saccharification) for bioethanol production involves two steps (YSI 2700 Select, Serial No. 06E0116, YSI Inc., Yellow Springs, OH), namely:

(1) enzymatic hydrolysis (saccharification);

$$\underbrace{(C_6H_{10}O_5)_n}_{\text{Cellulose}} + nH_2O \xrightarrow{\text{cellulases}} \underbrace{nC_6H_{12}O_6}_{\text{Glucose}} \quad (5.1)$$

and (2) fermentation.

$$\underbrace{C_6H_{12}O_6}_{\text{Glucose}} \xrightarrow{\text{microbes}} \underbrace{2C_2H_5OH}_{\text{Ethanol}} + 2CO_2 \quad (5.2)$$

These two steps could be done separately or in a combined state, frequently referred to as simultaneous saccharification and fermentation (SSF) (Wingren et al. 2003; Alfani et al. 2000). In this method, glucose is fermented into bioethanol in-situ without the need for extra separate reactor. However, the optimum operating conditions for enzymes and fermenting microorganism are generally different (Jørgensen et al. 2007). Cellulase have an optimum operating condition of approximately 50°C, whereas yeast and bacteria have growth optimal condition of around 32-37°C (Chen and Jin 2006; Wingren et al. 2003; Alfani et al. 2000). Therefore, there is a tradeoff between cost and optimum activity, because the operation of SSF is at suboptimal conditions for biomass enzymatic hydrolysis (Holtzapple et al. 1990; Jørgensen et al. 2007). Ethanol and lactic acid are inhibitory to cellulase. Such effects of enzymatic inhibition via fermentation byproducts must be taken into consideration and assessed before selecting the combined steps of hydrolysis and fermentation approach for the biorefinery industry (Chen and Jin 2006; Wingren et al. 2003; Alfani et al. 2000; Holtzapple et al. 1990).

The focus of this current investigation is on the enzymatic saccharification/hydrolysis of pretreated and non-treated biomass barley straw grind. In the previous findings (Iroba et al. 2013

and 2014), it was observed that the use of RF-alkaline and steam explosion (SE) pretreatment led to higher total acid soluble lignin moieties, with corresponding cellulose and hemicellulose yield. If the acid soluble lignin were easily accessed, it therefore implies that there was disruption and deconstruction of the lignified cross-linking matrix. It was predicted that this will enhance the accessibility and digestibility of the energy potentials (cellulose and hemicellulose) during the next stage of the process (enzymatic saccharification/hydrolysis), and subsequently assist in decreasing the required amount and costs of enzymes by up to a maximum of 64% (Iroba et al. 2013). Therefore, the objective of this investigation was to validate and evaluate the readily available enzyme digestible glucose (cellulose) in the RF-alkaline and SE pretreated and non-treated samples. Cellulose (glucose) is the major sugar (>40%) in barley straw, therefore; the yields of this sugar was used to assess the effectiveness of the pretreatment process.

5.3 Materials and Method

5.3.1 Sample Preparation and RF and SE Pretreatments

Barley straw ground in a hammer mill with a 1.6 mm screen size was pretreated using RF and SE techniques as described in detail by Iroba et al. (2013, 2014). The samples were dried and adjusted to 10% moisture (w.b.) using the forced-air convection dryer (Shaw et al. 2007) set at 40^{o}C. The chemical compositions (structural carbohydrates and the complex phenolic polymer (lignin)) of the pretreated and non-treated samples were analyzed using the Ultra Performance Liquid Chromatography (UPLC- Acquity 2004-2010, Waters Corp., Milford, MA) based on National Renewable Energy Laboratory standard (NREL- Sluiter et al. 2007). The NREL standard uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The RF and SE treatment combinations that resulted to the optimum yield of the total acid soluble lignin, cellulose, and hemicellulose were selected (Table 5.1) for enzymatic saccharification, and subsequently subjected to glucose analysis using the National Renewable Energy Laboratory Standard (NREL- Selig et al. 2008). This procedure is used for the enzymatic saccharification of cellulose from native or pretreated lignocellulosic biomass to glucose in order to determine the maximum extent of digestibility possible. This standard is also used to measure the efficacy of a given pretreatment based on a maximum enzyme loading. The particle size of the selected chemically and thermally altered samples (with changed structural composition) and

some selected pretreated densified samples (Table 5.1) were further reduced to 0.8 mm using the precision grinder (Falling Number, Model No. 111739, S-141 05 Huddinge, Sweden).

Table 5.1 Selected optimum RF alkaline and SE pretreated samples

Selected RF alkaline pretreated samples	Selected steam explosion pretreated samples		
Temperature (^{o}C)	Temperature (^{o}C)	Moisture content (%)	Retention time (min)
24	180	8	5
24[d]	180	8	10
70	180[d]	8[d]	10[d]
80	140	30	10
80[d]	160	30	10
90	160[d]	30[d]	10[d]
-	140	50	10

d = pretreated densified samples; RF optimal treatment combination was ratio 1:6 (100 g biomass and 600 g NaOH solution).

5.3.2 Washing of the RF-Alkaline Pretreated and Pretreated Densified Samples

According to National Renewable Energy Laboratory Standard (NREL- Selig et al. 2008), this method of enzymatic saccharification of lignocellulosic biomass is not suitable for acid- and alkaline-pretreated biomass samples that have not been washed. Unwashed pretreated biomass samples containing free acid or alkali may change solution pH to values outside the range of enzymatic activity; and the unwashed glucose in the biomass may influence the final result. Therefore, to remove the NaOH solution in the RF alkaline pretreated and densified pretreated samples, a vacuum filter consisting of a perforated porcelain funnel 200 mL with Whatman filter paper (size 40) was used. Tap water (approximately 1 L) was repeatedly used for the washing process until the pH reached around 7. The washed samples were again dried to 10% moisture (w.b.) using the forced-air convection dryer set at 40^{o}C. Prior to the enzymatic saccharification, the biomass straw at 10% (w.b.) was bone-dried at 105^{o}C in an air oven (Shel-Lab model No. 1350F; Sheldon Manufacturing Inc., Cornelius, OR) for 24 h.

5.3.3 Enzymatic Saccharification/Hydrolysis of Lignocellulosic Biomass

Barley Straw Grind

Approximately 150 ± 0.5 mg of the oven bone-dried total biomass sample on a 105^{o}C dry weight basis was weighed and added to a 26 mL (7 dram) clear scintillation vial. To each vial, 5.0 mL of 0.1 M, pH 4.8 sodium citrate buffer and 100 µL of a 2% sodium azide solution were added. The biomass materials were completely mixed with buffer solution ensuring it to be fully moist at all times. It allows water to penetrate into capillary spaces breaking hydrogen bonds inside cellulose molecules and forcing apart microcrystallites (Jacobus 1997; Klyosov 1990). The sodium azide helps prevent the growth of organisms during digestion. Cellulase (C2730-50 mL, cellulase from *Trichoderma reesei* ATTC 26921, Sigma-Aldrich Co., St. Louis, MO) and β-glucosidase (C6105-50 mL, Novozyme® 188, Sigma-Aldrich Co., St. Louis, MO) enzymes of known activity/concentration, 189 FPU/mL and 483 pNPGU/mL (CBU/mL), respectively, were used for the digestion. The expected cellulase and β-glucosidase enzymes activity approximately equals 60 FPU/g and 64 *p*NPGU/g cellulose, respectively. The volume of the cellulase and β-glucosidase enzymes of known activity to be added to the substrate (biomass sample) were calculated based on the following factors: the theoretical cellulose content (X%) in the substrate, which have already been determined by cell wall analysis by Iroba et al. (2013 and 2014) using the National Renewable Energy Laboratory Standard (NREL- Sluiter et al. 2007), dry weight of the oven bone-dried total biomass sample (Y g), and the expected enzyme activity of cellulase and β-glucosidase (Z FPU/g).

Sample calculation using the non-treated biomass (control), with 42.51% theoretical cellulose.
The filter paper unit (FPU) of the cellulase enzyme:

$$0.4251\ (X) \times 0.15004\ g\ (Y) \times 60\ FPU/g\ (Z) = 3.8269\ FPU \tag{5.3}$$

Therefore, the volume (mL) of cellulase enzyme to be added to the substrate:

$$= \frac{3.8269\ FPU \times 1\ mL}{189\ FPU} \tag{5.4}$$

In the same manner, the volume of β-glucosidase enzyme to be added was determined based on the above approach. Thereafter, the amount of distilled water needed to bring the total volume to 10.00 mL was calculated and added appropriately to each vial. Marsden and Gray (1985) reported that moisture has three functions in enzymatic hydrolysis: (1) it swells the fiber and opens up the fine structure; (2) water molecules are added to the glucosidic bonds during hydrolytic cleavage; and (3) it provides a medium for the diffusion of enzymes and products. Based on the NREL method standard, all solutions and the biomass are assumed to have a specific gravity of 1.000 g/mL. Thus, with the example given above where 0.15004 g of control biomass was added to the vial, it was assumed to occupy 0.15004 mL. The enzymes are always added last since the reaction is initiated by the addition of enzyme. The reaction blank for the substrate (using non-treated biomass) was prepared with the buffer, water, and identical amount of substrate in 10.00 mL volume. Cellulase blank (no cellulase but contains β-glucosidase) and β-glucosidase blank (no β-glucosidase but contains cellulase) were prepared with buffer, water, and the identical amount of the enzyme. The vials were tightly closed, placed in a scintillation vial rack, and placed in a shaking incubator (Classic C24, serial No. 790860283, New Brunswick Scientific Co. Inc., Edison, NJ). The temperature was set to $50^{o}C \pm 1^{o}C$, which is the optimum operating condition for the enzymes. The incubation was done for 96 h with shaking/rotation at 250 rev/min, which is sufficient to keep solids in constant suspension throughout the incubation period for the release of soluble sugars from the samples. At the end of incubation, the sample was allowed to cool at room temperature. Samples were centrifuged at 14 rev/min for 5 min using a microcentrifuge (Bio-Rad Labs, Model 16K, Serial No. B812473, Hercules, CA). The supernatants were used for sugar measurement. A 1 mL plastic syringe was used to draw a representative sample from the vial. The representative sample was filtered through a 0.45 μm filter into a 1 mL vial for glucose analysis. Three replicates of each sample were performed.

5.3.4 YSI Glucose Biochemistry Analyzer

The D-glucose (dextrose) in the hydrolyzed samples was subsequently analyzed using the YSI biochemistry analyzer (YSI 2700 Select, Serial No. 06E0116, YSI Inc., Yellow Springs, OH). To ascertain the effectiveness and performance of the instrument, 5 mg/mL of cellulose (type 20, Sigmacell®, S3504-1KG, Batch No. 107K0150, Sigma-Aldrich Co., St. Louis, MO) and α-D-

glucose (Anhydrous 96%, 158968-3KG, Batch No. MKBB8469, Aldrich Chemistry Co., St. Louis, MO) were separately prepared and measured. YSI Select 2700 employs a steady state measurement methodology and is equipped with automatic self-calibration with a frequency of every five sample measurement. A substrate (dextrose) enters the sample chamber, stirred and diluted. The substrate then diffuses through a thin polycarbonate membrane material. The rate of the chemical reaction in YSI which is shown below is limited primarily by diffusion. It is based on the principle of immobilized oxidase enzymes that employs enzyme catalyzed reactions to ultimately produce hydrogen peroxide, which electrochemically oxidized at the platinum anode of an electrochemical probe. The following reaction occurs:

$$\text{Substrate} + O_2 \xrightarrow[\text{oxidase}]{} H_2O_2 + \text{Byproduct} \tag{5.5}$$

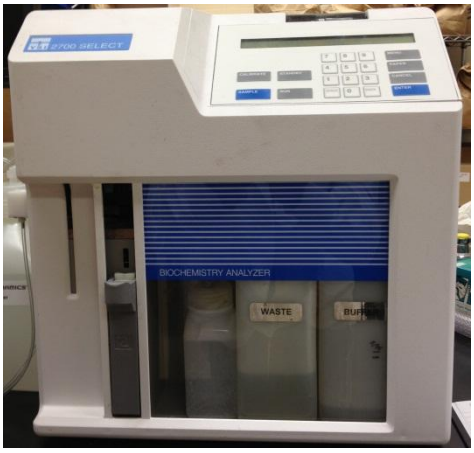


Figure 5.1 YSI 2700 Select glucose biochemistry analyzer.

This produces a probe signal current that subsequently translate into concentration of the glucose present in the sample. For each run of the analysis, the black and white membranes generate corresponding dextrose readings. Three replicates of each sample were performed.

5.3.5 Percentage Glucose Digestion/Proportional Glucose

The enzymes used (cellulase and β-glucosidase) are concentrations of the supernatant of the crude prepared enzymes. This implies that the enzymes are not 100% pure, therefore, the enzyme

blanks will contain traces of glucose concentration in the cellulase and β-glucosidase enzymes. Therefore, this trace of glucose concentrations in the centrifuged supernatant must be determined using the YSI by preparing enzyme blanks, and subsequently corrected/subtracted from the glucose concentrations of the substrates. To calculate the percent digestibility of the cellulose added to the scintillation vial, first the mass of cellulose digested (D) (g) was computed using equation 5.6:

D = YSI glucose reading, g/L (corrected with blanks) x V (mL) x C (5.6)

where C is equal to 0.9, the correction for hydration (to correct for the water molecule added upon hydrolysis of the cellulose polymer). V is equal to 10 mL (0.01 L), which is the total volume of assay, because the YSI Select 2700 uses only a representative quantity of about 25 µL for the glucose analysis.

The %digestion or proportional glucose was calculated using equation 5.7:

$$\%\text{glucose digestion} = \frac{\text{grams cellulose digested}}{\text{grams cellulose added}} \times 100 \quad (5.7)$$

The mass of cellulose added is the theoretical glucose (cellulose) obtained from the cell wall analysis using the UPLC based on the National Renewable Energy Laboratory standard (NREL-Sluiter et al. 2007).

The %digestion increase was computed based on equation 5.8:

$$\%\text{digestion increase} = \frac{\text{average \%glucose digestion of treated sample}}{\text{average \%glucose digestion of non-treated sample}} \text{ x } 100 \quad (5.8)$$

Assumption made: In the calculations using equations 5.3 and 5.7, it was assumed that the pretreated densified samples have the same amount of cellulose as the pretreated samples. This is because the chemical compositions of the densified samples were not analyzed using the UPLC after the densification process. This assumption will not affect the overall yield, but may influence the yield of the enzymatic hydrolysis, especially if more of the energy potentials have been further released during the densification process.

5.4 Results and Discussion

To determine and further investigate the suitability of RF alkaline and SE pretreatment of lignocellulosic biomass, the optimum treatment combinations with higher total acid soluble lignin, cellulose, and hemicellulose were selected. These were subjected to cellulose-based enzymatic hydrolysis to further breakdown the complex carbohydrate (cellulose) to simple sugar (glucose). The percentage glucose digestion or the proportional glucose as shown in Table 5.2 below validates the accessibility and digestibility of cellulose (glucose) in the RF alkaline and SE pretreated and non-treated samples. The obtained data reflects the efficiency and suitability of the pretreatment process. The average glucose concentration (impurity) present in the cellulase and β-glucosidase enzymes which was determined using the YSI was 0.01 g/l and 0.04 g/l, respectively. These were used for the correction of the substrate glucose concentration.

The average available glucose yield (%) that was released during enzymatic hydrolysis which is available for bioethanol production ranged from 78 to 96% for the RF-alkaline pretreated samples and 30 to 50% for the SE pretreated samples, depending on the treatment combination. The low percentage glucose yield from the SE samples reflects the severe initial degradation of cellulose (glucose) due to the carbonization that took place during the steam explosion process. The degradation was evaluated by performing elemental composition analysis of the SE pretreated and non-treated samples using the CHNS mode elemental analyzer vario EL III (Elementar Analysensteme GmbH, Serial No. 11014041, Hanau, Germany). This set up is a fully automatic instrument for a speedy and quantitative determination of CHNS (Carbon, Hydrogen, Nitrogen, and Sulfur, respectively). The elemental composition analysis showed an increase of elemental carbon content from 42.30% (non-treated) to about 46.69% (SE pretreated) depending on the treatment combination. For details of the degradation/carbonization and elemental composition analysis see Iroba et al. (2014). The degradation that resulted from the carbonization was also observed in the chemical compositions (acid soluble lignin and cellulose) as shown in Table 5.2. The RF alkaline pretreated samples have higher acid soluble lignin and cellulose relative to the SE pretreated samples. However, the RF alkaline and SE pretreated samples have higher acid soluble lignin and cellulose compared to the non-treated samples irrespective of the degradation of the SE pretreated samples. The non-treated sample has average available glucose yield of just below 12% due to the lignified bonded nature of non-treated lignocellulosic

biomass. Such low rate and extent of conversion inhibit the development of economically viable bioethanol production. Fan et al. (2006b) reported that the limited available sites for enzymatic reaction/attack is due to the fact that the average size of the capillaries in native/non-treated biomass is too small to permit the entrance or flow of large enzyme molecules, as such enzymatic attack is confined or limited to the external surface.

Table 5.2 Chemical composition and YSI glucose digestion of RF-alkaline and SE pretreated and non-treated barley straw grind

Pretreated temperature (^{o}C)	Moisture content (% w.b.)	Retention time (min.)	% Cellulose in substrate	Acid soluble lignin in substrate (ng/mg)	Average YSI glucose reading (g/L)	Average %glucose digestion	% Digestion increase over non-treated sample
Radio frequency pretreated samples							
Non-treated	8	-	42.51	286.82	0.86 (0.01)	11.34 (0.24)	-
24*	87	20	30.93	471.61	4.11 (0.07)	78.38 (1.26)	690.85
24^{d}*	87^{d}	20^{d}	30.93	-	5.00 (0.18)	95.76 (4.27)	844.07
70	87	20	28.25	419.45	4.79 (0.09)	96.64 (2.19)	851.85
80**	87	20	31.94	440.00	4.46 (0.09)	82.44 (1.90)	726.71
80^{d}**	87^{d}	20^{d}	31.94	-	4.89 (0.35)	96.44 (3.71)	850.10
90	87	20	30.37	375.68	4.71 (0.14)	91.91 (3.42)	810.16
Steam explosion pretreated samples							
180	8	5	9.58	322.10	0.53 (0.01)	30.16 (1.06)	265.86
180*	8	10	8.74	352.27	0.65 (0.01)	41.01 (1.07)	361.45
180^{d}*	8^{d}	10^{d}	8.74	-	0.78 (0.02)	50.23 (1.97)	442.80
140	30	10	15.38	302.35	0.87 (0.01)	32.13 (0.18)	283.21
160**	30	10	13.57	306.34	1.05 (0.01)	44.31 (0.80)	390.60
160^{d}**	30^{d}	10^{d}	13.57	-	1.07 (0.03)	44.89 (0.68)	395.73
140	50	10	16.02	303.10	1.02 (0.02)	36.31 (0.70)	320.09
Blank	8	-	42.51	286.82	0.12 (0.01)	0.97 (0.18)	-

d = pretreated densified sample; Value in parentheses is standard error; n = 2 for RF and SE pretreatment and chemical composition, n = 18 for YSI glucose digestion; Blank = substrate (non-treated biomass), buffer, and water with no enzymes; * and ** are samples assumed to have the same amount of cellulose.

In this investigation, the applied RF-alkaline pretreatment enhanced the degree of hydrolysis and

increased the glucose yield of barley straw grind 6.9 to 8.5 times (depending on the treatment combination) relative to the control (non-treated), while steam explosion pretreatment increased the glucose yield by 2.8 to 4.4 times compared to the control. There is usually low yield when non-treated/native lignocellulosic biomass is subjected to enzymatic hydrolysis due to the high stability of the material to enzymatic or bacterial attacks (Söderström et al. 2003). Fan et al. (2006b) reported that most often, biodegradation of non-treated lignocellulosic biomass results in low yield of sugar from enzymatic hydrolysis, generally below 20% of the theoretical maximum. This value (<20%) is comparable with the value of glucose yield (<12%) obtained from the non-treated biomass in this recent investigation. However, it is very difficult to compare such yield because of differences in feedstocks (chemical compositions), environmental effects, method of pretreatment, and data analysis approach. Kumar et al. (2009) investigated the enzymatic digestibility of corn stover that was pretreated with ammonia fiber explosion (AFEX) at different ratios of ammonia to biomass. The optimal pretreatment conditions for corn stover were found to be at temperature of 90°C, an ammonia/dry corn stover mass ratio of 1:1, moisture content of corn stover of 60% (dry weight basis), and residence time (holding at target temperature) of 5 min. These researchers reported that approximately 98% of the theoretical glucose yield was obtained during enzymatic hydrolysis of the optimally treated corn stover. The ethanol yield from the pretreated corn stover increased up to 2.2 times over that of the non-treated sample.

Sun and Cheng (2002) and Feist et al. (1970) reported that the mechanism of the interaction of alkaline and lignocellulosic biomass causes saponification of intermolecular ester bonds linkages within the biomass thus promoting the swelling of lignocellulosic biomass beyond water-swollen dimensions and favoring increased enzymatic and microbiological penetration into the cell-wall fine structure. Millett et al. (1975) using alkali-treated hardwood and Fan et al. (1981c) with wheat straw pretreated by different methods including both acid and alkali, reported that hydrolysis of the cellulose increased rapidly with the removal of lignin. Chosdu et al. (1993) used a combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark, and peanut husk. The glucose yield of corn stalk was 20% in non-treated samples compared to 43% after treatment with electron beam irradiation at a dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively. Azzam (1989) investigated the pretreatment of cane bagasse with hydrogen peroxide. This researcher

reported that hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% of the lignin and most of the hemicellulose were solubilized by 2% H_2O_2 at 30°C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45°C for 24 h. Bjerre et al. (1996) used wet oxidation and alkaline hydrolysis of wheat straw (20 g of straw/L, 170°C, 5-10 min) and achieved an 85% conversion yield of cellulose to glucose.

Based on equations 5.3 and 5.4, the non-treated biomass consumed higher amount of enzymes than the RF-alkaline and SE pretreated samples (Table 5.3). Even with the higher amount of enzymes added to the non-treated biomass, the available glucose yield was still lower than the RF-alkaline and SE pretreated samples. This implies that more enzymes are required in order to increase the available glucose yield in the non-treated biomass. More enzymes imply more cost for the biofuel industry. Cost is one of the major challenges that the biofuel industry is experiencing today. Li et al. (2004) and Mais et al. (2002) reported that pretreatment of biomass by ball milling the particles enhanced the rate of saccharification and decreased the amount of enzyme required to achieve total hydrolysis of the carbohydrate moieties. Mais et al. (2002) reported about 70% conversion efficacy of α-cellulose saccharification using simultaneous ball milling and enzymatic hydrolysis at 2.5% (w/v) and 25 FPU/g of cellulose with retention time of 48 h.

Table 5.3 Amount of cellulase and β-glucosidase enzymes used for the enzymatic saccharification of RF-alkaline and SE pretreated and non-treated lignocellulosic biomass barley straw grind

Radio frequency pretreated samples				
Pretreated temperature (^{o}C)	Moisture content (%)	Retention time (min.)	Amount of cellulase used (µL)	Amount of β-glucosidase used (µL)
Non-treated	8	-	20.24	8.45
24*	87	20	14.73	6.15
24^{d}*	87^{d}	20^{d}	14.73	6.15
70	87	20	13.45	5.62
80**	87	20	15.21	6.35
80^{d}**	87^{d}	20^{d}	15.21	6.35
90	87	20	14.46	6.04
Steam explosion pretreated samples				
180	8	5	4.56	1.90
180*	8	10	4.16	1.74
180^{d}*	8^{d}	10^{d}	4.16	1.74
140	30	10	7.32	3.06
160**	30	10	6.46	2.70
160^{d}**	30^{d}	10^{d}	6.46	2.70
140	50	10	7.63	3.18

d = pretreated densified sample; * and ** are samples assumed to have the same amount of cellulose.

This investigation confirms that the pretreatment of biomass subsequently assisted in decreasing the required amount and costs of enzymes for the bioethanol industry. Vega et al. (1991) and Li et al. (2004) reported that high enzyme loading will significantly increase cost and use of high enzyme concentrations may not be economically justified.

The reaction blank for the substrate as shown in Table 5.2 depicts the effectiveness of the enzymes used. There was a significant decrease in the available glucose yield of the reaction blank relative to the non-treated enzymes hydrolyzed sample. The densification of biomass seemed to be a further pretreatment process. The densified biomass had higher available average percentage glucose yield that was released during the enzymatic hydrolysis. This may have been due to the compaction/compression of the biomass at the preset densification temperature, which may have caused further deconstruction/disruption of the biomass matrix. From this investigation, it is being hypothesized that compression/densification/pressing alters the biomass

physical and chemical structures and further melt and caused re-distribution of alkaline within the cell cavities of the biomass, leading to further breaking down of the biomass bonds during the densification process. Consequently, higher hydrolysis yield of soluble sugar (glucose) results. However, the information/data obtained from this current investigation is not a sufficient evidence to conclude this trend; as such further investigation is required in future to ascertain this preliminary finding/investigation.

5.5 Conclusion

In this investigation, enzymatic hydrolysis of RF-alkaline and SE pretreated and non-treated lignocellulosic biomass barley straw grind was performed. Based on the data collected, it is evident that pretreatment fractionate lignin and hemicellulose, leaving a low molecular mass cellulose. RF alkaline and SE pretreatment techniques create reaction sites for enzymatic hydrolysis with a corresponding higher glucose yield relative to the non-treated sample; however, RF alkaline technique is more efficient than the SE. The RF alkaline pretreated samples has higher acid soluble lignin, percentage cellulose and glucose digestion yield compared to the SE pretreated samples. This is due to the initial carbonization effect (with elemental carbon content increase from 42.30% to about 46.69%) that resulted to the severe degradation of the energy potentials (cellulose and hemicellulose) during the SE process. The pretreated samples required low enzyme loading with a corresponding higher glucose yield unlike the non-treated sample. The glucose yield shows the amount of readily available glucose in the RF alkaline and SE pretreated and non-treated biomass that can be released during the enzymatic hydrolysis, which will be available for bioethanol production. The yield in RF alkaline pretreatment was better correlated with the ratio of biomass to NaOH solution followed by the RF temperature. Based on the data collected from this present investigation, the most favorable pretreatment condition from the RF alkaline technique is ratio 1:6 (biomass to NaOH solution), 70^{o}C, 20 min retention time, 1% NaOH, with a hammer screen size of 1.6 mm. While the optimum condition for the SE pretreatment was identified to be at 160^{o}C, 30% moisture content, and 10 min retention time with a hammer screen size of 1.6 mm. Therefore, it can be concluded that to improve the yield of lignocellulosic biomass for bioethanol production, it is necessary to perform pretreatment prior to the enzymatic hydrolysis step.

Chapter 6

6. Application of Fourier Transform Infrared-Photoacoustic Spectroscopy for the Compositional Analysis of Radio Frequency-Alkaline and Steam Explosion Pretreated and Non-Treated Barley Straw

A similar version of the first half of this chapter (sections 6.4.3-6.4.4) has been and will be presented at the Advanced Biofuels Symposium, Ottawa, May 27-29, 2014 and CSBE/ASABE Meeting, Montreal, Quebec, July 13-16, 2014, respectively:

- Iroba, K.L. and L.G. Tabil. 2014. Rapid chemical compositional analysis of biomass for the biofuel industry. 2014 Advanced Biofuels Symposium/Annual General Meeting, Ottawa. BFN Project Number 29. May 27-29.

- Iroba, K.L. and L.G. Tabil. 2014. Application of Fourier transform infrared-photoacoustic spectroscopy for the compositional analysis of radio frequency alkaline pretreated and non-treated barley straw. 2014 Joint American Society for Agricultural and Biological Engineers (ASABE)/Canadian Society for Bioengineering (CSBE) Meeting, Montreal , Quebec. Paper Number 1897850. July 13-16.

A similar version of the preliminary results of this chapter has been presented as a poster at the BiofuelNet Annual General Meeting, Montreal, Quebec:

- Iroba, K.L. and L.G. Tabil. 2013. Characterization of chemical composition of radio frequency alkaline, steam explosion pretreated, and non-treated lignocellulosic biomass

using Synchrotron Radiation-FTIR-PAS. BiofuelNet Annual General Meeting, Montreal, Quebec. BFN Project Number 29. June 19-21, 2013.

Contributions of Ph.D. Candidate

A detailed review and optimization studies was conducted to further evaluate the potential applicability of infrared spectroscopy to the biofuel industry. The superior spectral resolution associated with Fourier transform infrared (FTIR) spectroscopy can be used to determine the chemical composition distribution and the structural characteristics of lignocellulosic biomass. Subsequently, an innovative procedure to rapidly quantify the lignocellulosic composition of radio frequency alkaline, steam exploded and non-treated barley straw grind was developed. This procedure could be easily adopted by the biofuel industry and extended to any form of lignocellulosic biomass using FTIR spectroscopy. The experiments, data analysis and manuscript writing were performed by Kingsley Iroba, while Dr. Tabil provided input in terms of analysis of the results and editing of the manuscript. Dr. Ferenc Borondics of Canadian light Source (Synchrotron) provided technical support during the conduct of the experiment. The FTIR-photoacoustic spectroscopic experiment was performed at the Canadian Light Source (Synchrotron), Saskatoon, SK.

Contributions of this Paper to Overall Study

Knowledge Gap: The use of the traditional approach in the quantitative analysis of biomass components (cellulose, hemicellulose, and lignin) is often performed using acid hydrolysis followed by analytical and gravimetric determination. This approach is complicated and time consuming. Therefore, the objective of this present investigation is to study and rapidly estimate the relative chemical composition of lignin, cellulosic and hemicellulosic polymers in pretreated and non-treated lignocellulosic barley straw using Fourier transform infrared-photoacoustic spectroscopy, and also to develop an easy and non-laborious approach for the quantitative measurement of lignocellulosic biomass chemical compositions.

Justification: Lignocellulosic biomass preprocessing and pretreatment techniques have demonstrated to improve the porosity, density, durability, dimensional stability of the produced pellets. These are primarily attributed to the structural and compositional modifications in the distributions of the lignocellulosic components, such modifications include: the surface area, the crystallinity of the cellulose, degree of polymerization, and the lignin seal surrounding the cellulose fibers, which lead to the structural resistance of cellulose (Sokhansanj et al. 2005; Fan et al. 2006a; Jeoh et al. 2007; Fan et al. 2006b). Hence, it is of paramount importance to have an in-depth understanding of lignocellulosic matrix at the molecular level. FTIR is associated with superior spectral resolution and provides information on the fundamental molecular vibrations (Gordon et al. 2011). This permits better discrimination of structural and compositional differences, and often better structural interpretation (Gordon et al. 2011). Furthermore, FTIR analysis requires only small amounts of biomass plant material, which helps when screening samples available in limited quantities (Gordon et al. 2011). FTIR has the potential to produce quantitative data at high speed and throughput with minimum or no sample preparation (Budevska 2002). The conventional approach (acid hydrolysis followed by analytical and gravimetric determination) of biomass chemical compositional analysis is highly time consuming, laborious and costly to perform (Pandey 1999; Adapa et al. 2011b; Gordon et al. 2011). Therefore, it is necessary to rapidly quantify and evaluate the modification in the compositional and structural arrangement of cellulose-hemicellulose-lignin matrix resulting from pretreatment.

6.1 Abstract

Lignocellulosic biomass has been identified as a potential feedstock for the biofuel industry. Quantitation of biomass components (lignin, cellulose, and hemicellulose) is often performed using the traditional acid hydrolysis followed by gravimetric determination. This approach is complicated and time consuming. In this study, barley straw grind was pretreated using radio frequency-alkaline and steam explosion techniques. The effect of the pretreatment was first evaluated using the traditional approach. Then, Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) was used in light of the need for rapid and easy quantification of biomass components. The effect of the treatment was measured based on their photoacoustic

(PA) spectra. In order to develop a predictive model that will be swiftly used for the quantitative prediction of the chemical composition of the biomass, reference materials: pure cellulose (microcrystalline powder), hemicellulose (xylan from birch wood), and lignin (hydrolytic) powders were mixed in different proportions with known concentrations. The reference materials were used to generate standard spectra to determine the relationship between the respective quantity of components in the mixture and the FTIR spectra of representative biomass sample. The FTIR wavenumber-dependent instrumental effects were corrected by using carbon black reference spectrum. Multiple linear regression models for cellulose, hemicellulose, and lignin were developed based on the generated regression parameters, with coefficient of determination 0.68, 0.34, and 0.31, respectively, and mean square error of 9.10, 13.86, and 15.86, respectively. The results obtained show that the FTIR-PA spectra can be rapidly used for biofuel feedstock identification and analysis of the chemical composition unlike the traditional approach.

6.2 Introduction

Agricultural biomass residue is a naturally occurring polymeric compound comprised of cellulose, hemicellulose, lignin, and extractives (Pandey 1999). Biomass has the potential to be used as raw material for the biofuel industry (Adapa et al. 2009). Cellulose occurs as crystalline fibers with random amorphous portions. The crystallinity of cellulose fibers is a key obstacle for effective biomass enzymatic hydrolysis (Ramesh and Singh 1993). Hemicellulose functions as a matrix for the cellulose and acts as a linkage between the crystalline cellulose and amorphous lignin (Ramesh and Singh 1993; Pandey 1999). Lignin is a polyphenolic compound that acts as a cementing material for biomass (Pandey 1999). Lignin binds the hemicellulose and cellulose within the matrix. It provides rigidity and mechanical strength, holding it together (Iroba and Tabil 2013a). The complex structure of lignocellulosic biomass presents resistance and 'recalcitrance' to biological and chemical degradation during enzymatic hydrolysis/saccharification and the subsequent fermentation process. This leads to a very low conversion rate, which makes the process less economically feasible (Söderström et al. 2003; Fan et al. 2006a; Chandra et al. 2007; Mohammad and Karimi 2008). Pretreatment strategies are employed to overcome the aforementioned challenges by deconstructing/disrupting the

lignocellulosic matrix (Iroba et al. 2013b), while making cellulose and hemicellulose accessible for the production of bioethanol**.**

Radio frequency-alkaline and steam explosion pretreatment of biomass barley straw have shown that pretreatment enhanced the modification of the natural binding characteristics of lignocellulosic biomass (Iroba et al. 2013b; Iroba et al. 2014a). Subsequently, this improved the physical properties of the produced pellets from the pretreated biomass (Iroba et al. 2014b).
The traditional methods of determining the chemical compositions of biomass involve gravimetric and analytical procedures. These procedures are time-consuming, laborious and expensive to perform, with low sample throughput and often results in a corresponding degradation of natural polymers (Pandey 1999; Adapa et al. 2011b; Gordon et al. 2011). In contrast, procedures involving infrared spectroscopy are useful tools in rapidly extracting information about the structure of biomass constituents and the associated chemical changes resulting from various biomass treatments (Pandey 1999). Fourier transform infrared (FTIR) spectroscopy has been successfully applied in a variety of species in wood surface characterization, for estimating the carbohydrate and lignin components (Pandey 1999; Gordon et al. 2011). FTIR is associated with superior spectral resolution and provides information on the fundamental molecular vibrations (Gordon et al. 2011). This permits better discrimination of structural and compositional differences, and often better structural interpretation (Gordon et al. 2011). Furthermore, FTIR analysis requires only small amounts of biomass plant material, which helps when screening samples available in limited quantities (Gordon et al. 2011). Applications of FTIR have also been found in biomedical weld and medicine research such as cancer and bone (Diem et al. 2004; Huang et al. 2003).

Changes in plant cell wall composition following pretreatment largely influence the end use performance. The ability to correctly and easily monitor these compositional changes is, therefore, important. Hence, the objective of this investigation is to study and rapidly estimate the relative chemical composition of lignin, cellulosic and hemicellulosic polymers in pretreated and non-treated lignocellulosic barley straw using FTIR-PAS. This work focuses on the effects

of radio frequency- alkaline and steam explosion pretreatments on the cell compositions of barley straw.

6.3 Material and Methods

6.3.1 Material Procurement and Preparation

Barley straw of the "Xena" variety was grown in Maymont, SK (56.667°N, 107.794°W) and obtained from RAW Ag Ventures Limited (Maymont, SK) in October 2009. To increase the surface area of the biomass, the straw was ground using a hammer mill (Model No. GM13688, Glen Mills Inc., Maywood, NJ) with screen size of 1.6 mm. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation and to provide flow of the biomass in and out of the hammer mill. The initial moisture content of the straw was 8.09% (wet basis). The moisture content was measured based on ASABE standard method, ASAE S358.2 (2008). As a comparison between pretreatment methods, the 1.6 mm biomass grind was subjected to two different pretreatment methods: radio frequency (RF)-alkaline pretreatment using a RF machine (1.5 kW & 27.12 MHz laboratory dryer, Strayfield, Theale, Reading) in a blown glass reactor (volume 4.25 liters) stationed in University of Saskatchewan, Saskatoon, SK, and steam explosion (SE) pretreatment located at the Clean Energy Research Center, University of British of Columbia, Department of Chemical and Biological Engineering, Vancouver, BC. The material and operating variables considered in both methods of pretreatment are shown in Table 6.1 (a-b). Each pretreatment was performed in two replicates.

Table 6.1a RF-alkaline material and operating variables using blown glass reactor

Variables	Levels
Hammer screen size	1.6 mm
NaOH solution concentration	1% w/v
Biomass: NaOH solution ratio	1:4 - 110 g biomass and 440 g NaOH solution 1:5 - 110 g biomass and 550 g NaOH solution 1:6 - 100 g biomass and 600 g NaOH solution 1:7 - 100 g biomass and 700 g NaOH solution 1:8 - 90 g biomass and 720 g NaOH solution
Soaking time	1 h
Temperature	70, 80, and 90^{o}C
Residence time	20 minutes

Table 6.1b SE material and operating variables with corresponding levels

Variables	Levels
Temperature (^{o}C)	140, 160, and 180
Moisture Content (% mass fraction of water)	8, 30, and 50
Retention Time (min.)	5 and 10

6.3.2 Chemical Composition Analysis of Lignocellulosic Biomass

The chemical composition analysis of the RF-alkaline and SE pretreated biomass grind was performed using the National Renewable Energy Laboratory standard (NREL) (Sluiter et al. 2007) at a laboratory facility at the Agriculture and Agri-Food Canada, Saskatoon, SK. Each sample was replicated twice. The NREL standard uses a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The first step uses 72% H_2SO_4, while the second step uses 4% H_2SO_4. The lignin fractionates into acid insoluble and acid soluble material. The acid insoluble lignin is the residue (remaining solids) from the hydrolysis suspension. Acid-soluble lignin moieties were quantified using the Waters Acquity Ultra Performance Liquid Chromatography–MS system (Acquity 2004-2010, Waters Corp., Milford, MA), which has the capability of separating and quantifying the various lignin components. The complex carbohydrates are hydrolyzed into monomeric forms (xylose, arabinose, mannose, glucose, and galactose) and subsequently quantified using UPLC-MS. The percentage hemicellulose was obtained by adding up the percentage xylose, arabinose, mannose, and galactose, while the percentage glucose was assigned to percentage cellulose. Further details on the material preparation, physical characteristics of the biomass grind, radio frequency alkaline technique, steam explosion process, and the chemical composition analysis using the NREL standard can be obtained in the research studies of Iroba et al. (2013b) and Iroba et al. (2014a). The pretreated and non-treated samples were further ground to screen size of 0.354 mm using a precision grinder (Falling Number, Model No. 111739, Huddinge, Sweden).

6.3.3 Preparation of Reference Materials of known Concentration

In order to develop a predictive model that will be rapidly used for the quantitative prediction of the chemical composition contained in the RF-alkaline and SE pretreated biomass, pure cellulose

(microcrystalline powder), hemicellulose (xylan from birch wood), and lignin (hydrolytic) powders (Sigma-Aldrich Canada Ltd., St. Louis, MO) were mixed in different proportions (Table 6.2). These were used as reference spectra to determine the relationship between the respective quantity in the mixture and the representative sample FTIR spectra. The FTIR wavenumber-dependent instrumental effects were corrected by using carbon black reference spectrum.

Table 6.2 Reference materials: Pure cellulose, hemicellulose, and lignin mixtures used to obtained the reference spectra (adapted from Adapa et al. 2011b)

Reference Mixtures	Cellulose (%)	Hemicellulose (%)	Lignin (%)
C1H0L0	100	0	0
C0H1L0	0	100	0
C0H0L1	0	0	100
C7H2L0	75	25	0
C2H7L0	25	75	0
C0H2L7	0	25	75
C0H7L2	0	75	25
C7H0L2	75	0	25
C2H0L7	25	0	75
C5H2L2	50	25	25
C2H5L2	25	50	25
C2H2L5	25	25	50
C3H3L3	33	33	33
Total	**13**	**13**	**13**

C, H, and L represent Cellulose, Hemicellulose, and Lignin, respectively

6.3.4 Fourier Transformed Infrared Photoacoustic Spectroscopy (FTIR-PAS)

Intensity of spectra generally increases as the particle size decreases (Michaelian 2003). To avoid moisture interference, the biomass samples were further dried using the forced-air convection dryer (Shaw et al. 2007) set at 40^{o}C for 48 h. Photoacoustic intensities are lower for samples with high moisture content. This might be due to lower efficiency of heat transfer between the moist cellulose surface and the carrier gas (Gurnagul et al. 1986). The Infrared data/spectra of the reference materials and biomass samples were collected using Mid-IR beamline (01B1-1) with energy range of 4000-400 cm^{-1}, at the Canadian Light Source Inc. (CLS, University of Saskatchewan, Saskatoon, SK). The beamline has a MTEC Model 300 photoacoustic cell (MTEC Photoacoustic Inc., Ames, IA) for FTIR-PAS of bulk samples. The sample cup was

filled with reference biomass sample (52-75 mg, depending on the pretreatment type and combination) and purged with helium gas to remove water vapor and CO_2. Helium gas is also needed in the medium because of it sound propagating properties. The collected FTIR spectra of the reference materials and biomass samples were recorded using Globar source (silicon carbide rod). When the radiation is incident on the sample, the energy of the Infrared beam is being absorbed by the sample layer. The photoacoustic signal is generated by thermal expansion of the gas caused by heat associated with the thermal wave emanating from the sample. The photoacoustic signal is carried by a carrier gas (Helium) to a microphone which is transferred to the FTIR electronics (detector) for processing; this ultimately produces the needed spectrum (McClelland et al. 2002; McClelland et al. 1992). The spectrum for each reference material and biomass samples were recorded separately averaging 64 interferograms (number of scans) collected from wavenumbers of 2000-400 cm^{-1} at a resolution of 4 cm^{-1}. The higher the number of scans the better the signal (lesser noise). Stuart (1997) reported that the signal-to-noise ratio (SNR) is proportional to the square root of the number of scans, n (SNR α $n^{0.5}$). Therefore, the higher the number of scans, the higher is the SNR. Resolution of 4 cm^{-1} was used to be able to discriminate between too close overlapping peaks, help increase the SNR and subsequently obtain higher resolution. The OPUS 6.5 (Brucker Optics Inc. Billerica, MA) software was used for the collection of the FTIR-PAS spectra. Three replicates were performed for each reference and biomass samples.

6.3.5 Determination of Concentration

Beer-Lambert discovered that the amount of light transmitted by a solid sample was dependent on the thickness of that sample (Stuart 1997). The Beer-Lambert law which can be applied to all electromagnetic radiation, states that the absorbance of a material is directly proportional to the thickness and concentration of the sample as shown: $A = \text{Ɛ}CL$. A = absorbance of the material, C = concentration, L = pathlength of the sample, and Ɛ = constant of proportionality, which is referred to as the molar absorptivity (Stuart 1997). Infrared spectra, particularly in the spectra of solid samples are often associated with the presence of asymmetric bands. As such, peak height cannot be used for the quantitative analysis of the spectra, because the baseline will vary from sample to sample. Instead, peak-area measurements should be used (Stuart 1997).

6.3.5.1 Quantitative and Qualitative Analysis of the FTIR-PAS Spectra

The two quantities of greatest interest in virtually any type of spectroscopy are, of course, band positions (wavenumbers) and intensities, the former generally conveying qualitative information, the latter quantitative (Michaelian, 2003). Therefore, these two variables were used for the FTIR data analysis.

6.3.6 Spectrum Manipulation

There are techniques that assist in both qualitative and quantitative interpretation of spectra. OriginPro software (Data analysis and graphing Version 8.6, OriginLab Corporation Northampton, MA) was used for the spectrum manipulation, quantitative, and qualitative analysis of the FTIR-PAS Spectra:

6.3.6.1 Baseline Correction, Subtraction, and Rescaling

The Baseline Mode is a tool for choosing a baseline mode and creates the baseline. A user-defined baseline treatment was applied in this analysis. A common flat baseline of 0.012, joining the points of lowest absorbance (via fitting Pro) on the peak was selected and applies across all the reference materials and biomass sample spectra. Thereafter, baseline subtraction was performed from the input data, such that the absorbance difference between the selected baseline and the top of the band is then used. This helps to improve the accuracy of the peak finding. Rescaling of the baseline to zero was subsequently performed, so that all the spectra will have a common origin of zero.

6.3.6.2 Smoothing

Smoothing is a signal processing technique typically used to remove or diminish noise from signals/spectrum. After a spectrum is smoothed, it becomes similar to the result of an experiment obtained at a lower resolution (Stuart 1997; OriginLab Corporation manual). The features are blended into each other and the noise level decreased. A smoothing function is basically a convolution between the spectrum and a vector whose points are determined by the degree of smoothing one wish to apply (Stuart 1997). There are multiple smoothing methods that work differently depending on the nature of the signal and the noise contained in the signal. Each method offers a different performance. In this present analysis, Savitzky-Golay was used. The

Savitzky-Golay filter method performs a local polynomial (order of 2) regression around each point, and creates a new, smoothed value for each data point. This method is superior to other methods (such as adjacent averaging) because it tends to preserve features of the data, such as peak height and width, which can be "washed out" by adjacent averaging (OriginLab Corporation, Northampton, MA, 2012). To increase the smoothness of the result, one can increase the "window size," used in each local regression (OriginLab Corporation, Northampton, MA, 2012). As such, the window size in this analysis was increased from one to two. But for very large window size, adjacent averaging may depart from the input signal too much, whereas Savitzky-Golay can still preserve the overall profile (OriginLab Corporation, Northampton, MA, 2012).

6.3.6.3 Peak Finding Settings

Manual peak editing was performed so as to effectively select the required respective peaks. The second (2^{nd}) derivative was used to search for all the hidden peaks and heavily overlapped bands included in the spectrum data. Differentiation is used to resolve and locate peaks in an envelope. Sharp bands are enhanced at the expense of broad ones, and this may allow easier selection of a peak, even when there is a broad band beneath it (Stuart 1997).

6.3.6.4 Characteristic Peak Assignment

The characteristic wavenumbers for pure cellulose, hemicellulose, and lignin listed in Table 6.3, were used for the peak assignment. Five characteristic peaks were identified for the pure cellulose, six characteristic peaks for pure hemicellulose, and six characteristic peaks for pure lignin (Figure 6.1a-c). The number of the peaks identified for the respective reference materials depends on the mixture of the reference materials. All seventeen peaks were identified for the treated and non-treated biomass samples.

6.3.6.5 Peak Integration

To obtain quantitative values from the area under the manipulated spectrum/peaks, the area under the respective peaks were integrated and output into excel file.

6.3.6.6 Normalization of Photoacoustic Infrared Spectra

Prior to the spectrum manipulation, the FTIR-PAS biomass sample spectra were corrected for wavenumber-dependent instrumental effects by dividing the reference carbon black ("background") spectrum intensity. This strategy implicitly assumes that the stability of the instrumentation used is adequate to ensure reliable results, even though the sample and reference spectra were collected at different times (Michaelian 2003). Carbon black is featureless, in the sense that it does not show any major characteristics peak (Michaelian 2003). Photoacoustic (PA) cell intensities varied with sample packing in the PA cell (St. Germain and Gray 1987). Stuart (1997), also reported that absorbance varies linearly with the sample thickness. Therefore, the effect of bulk density of the reference materials and biomass samples was corrected by dividing the integrated areas with respective mass of the reference materials and biomass samples contained in the PA sample cup.

The model was further standardized by normalizing the corrected integrated area data from 0 to 1. This was performed by dividing individual reference materials and biomass samples corrected integrated area data by corresponding maximum corrected integrated area data value. The aforementioned steps were performed for the three major components of lignocellulosic biomass (cellulose, hemicellulose, and lignin). Therefore, this normalization approach ensures that the predictive model is adaptable for quantitative analysis of FTIR-PAS spectra obtained for any lignocellulosic biomass.

6.3.7 Regression Analysis

This portion of the analysis is divided into two parts: the training and verification analysis:

6.3.7.1 Training Analysis

The normalized data of cellulose, hemicellulose, and lignin components in the reference materials and the combined biomass samples (i.e. combination of data from RF and SE analysis) were correlated to their percentage compositions. The RF and SE normalized data were combined so as to develop a general model that can be applied on a barley straw sample, irrespective of the pretreatment method applied on the biomass. The percentage compositions of the reference materials (Table 6.2) and the percentage compositions (Tables 6.4 & 6.6-6.8) of

the combined biomass samples obtained from the NREL Standard were used as the dependent variable, while two out of the three replicates from the combined normalized data (obtained from the PA spectra) were randomly selected and used as the independent variables. Five independent variables were used for the cellulose (because it has five characteristic wavenumbers), while six independent variables were used for both hemicellulose and lignin (they both have six characteristic wavenumbers each). Multiple linear regression analysis was conducted at 5% significance level using IBM SPSS Statistics (Superior Performing Statistical Software, version 20 for Windows, 2012; IBM, Armonk, New York, NY). Regression models (equations 6.1-6.3) for cellulose, hemicellulose, and lignin were developed based on the generated regression coefficients. The developed models have the capability to predict quantitatively, the percentage compositions of cellulose, hemicellulose, and lignin in any lignocellulosic biomass.

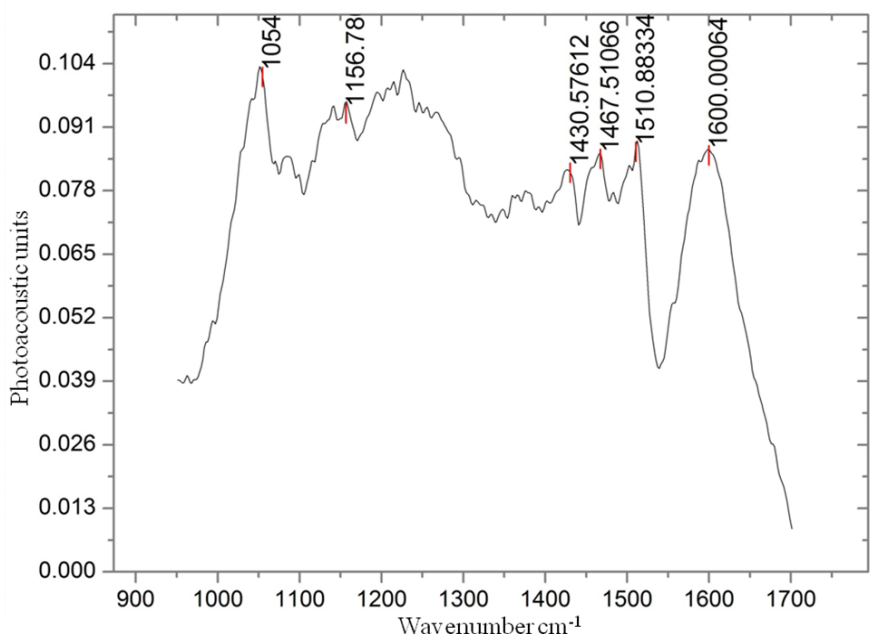
6.3.7.2 Verification Analysis (Validation)

Subsequently, the normalized data (based on the respective characteristic peaks) from the third replicate of biomass sample was substituted into the developed predictive models to estimate/predict the percentage compositions of lignocellulosic components present in the non-treated, RF-alkaline and SE pretreated biomass samples (Tables 6.4 & 6.6-6.8).

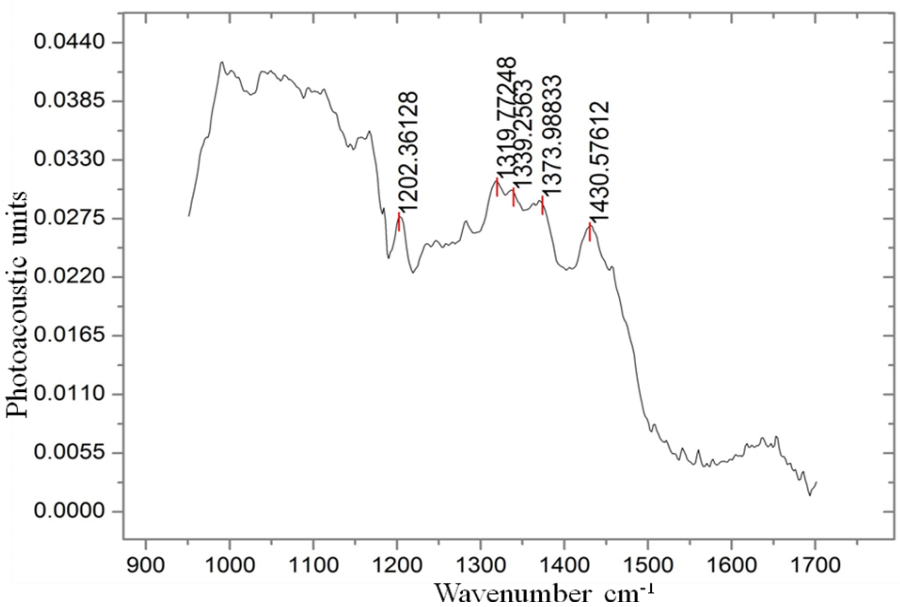
6.4 Results and Discussion

6.4.1 Reference Material Spectra

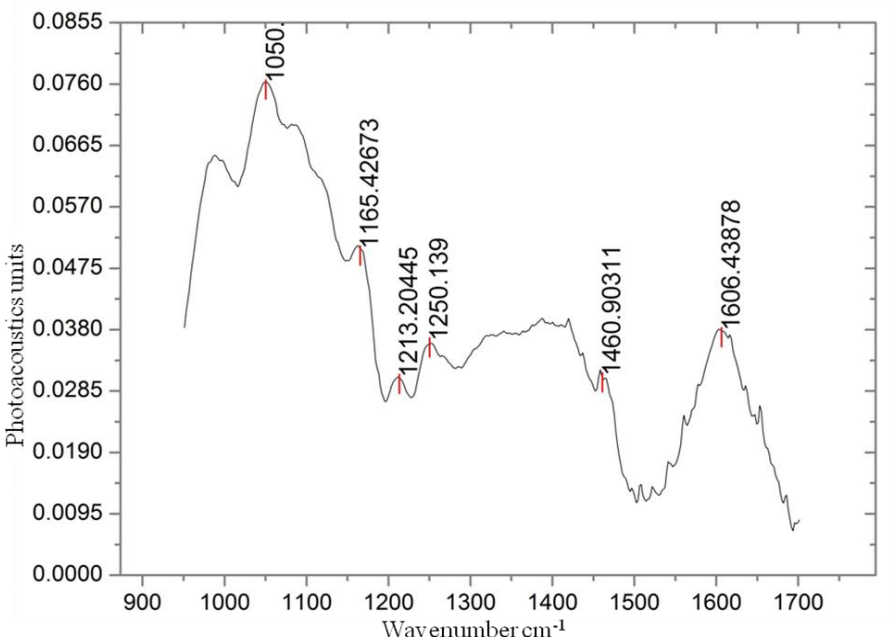
Figure 6.1 (a-c) shows the normalized spectral results obtained from a typical reference cellulose (100%), hemicellulose (100%), and lignin (100%), respectively, after smoothening and baseline corrections. Generally, FTIR-PAS techniques permit to obtain spectra which present specific characteristic key bands of individual components. In principle, such band position allows the discrimination of different species and provides important information about the chemical compositions of the material.



(a) Lignin



(b) Cellulose



(c) Hemicellulose

Fig. 6.1 FTIR-PAS spectra of a) pure lignin (hydrolytic), b) cellulose (microcrystalline powder), and c) hemicellulose (xylan from birch wood) powders showing prominent peaks/band positions at the various characteristics wavenumbers.

6.4.2 Characteristic Peak Positions

The various prominent (characteristic) peak (band) positions and the corresponding peak assignments for cellulose, hemicellulose, and lignin are presented in Table 6.3. These are distinct peaks at different wavenumbers associated with these three biomass components. Lignin spectrum has six prominent peaks at wavenumbers of 1599 (X_1), 1511 (X_2), 1467 (X_3), 1429 (X4), 1157 (X_5), and 1054 cm^{-1} (X_6) (Figure 6.1a). Cellulose spectrum has five distinct peaks at wavenumbers of 1431 (X_1), 1373 (X_2), 1338 (X_3), 1319 (X_4), and 1203 cm^{-1} (X_5) (Figure 6.1b). Hemicellulose spectrum has six characteristic peaks at wavenumbers of 1606 (X_1), 1461 (X_2), 1251 (X_3), 1213 (X_4), 1166 (X_5), and 1050 cm^{-1} (X_6) (Figure 6.1c).

Table 6.3 Characteristics peaks/bands position and assignment of pure cellulose, hemicellulose, and lignin (adapted from Adapa et al. 2011b)

Wavenumbers (cm^{-1})	Cellulose 100%	Hemicellulose 100%	Lignin 100%	Peak Assignment
1650-1600	-	1606	-	1604-acetylated hemicellulosic polysaccharides (Himmelsbach and Akin, 1998).
1600-1550	-	-	1599	1595-aromatic ring stretch, strongly associated with aromatic C-O stretching mode (Stewart et al., 1995); 1595-phenylpropanoid polymer (Himmelsbach and Akin, 1998); 1600-quadrant ring stretching (aromatic compound of lignin) (Colthup et al., 1990; Yu et al., 2007); 1600-aromatic skeletal vibration (Pandey, 1999).
1550-1500	-	-	1511	1515-semicircle ring stretching (aromatic compound of lignin) (Colthup et al., 1990; Yu et al., 2007); 1510-lignin aromatic compound (Yu, 2005); 1510-very strong aromatic ring stretch, aromatic C-O stretch (Revol, 1982; Stewart et al., 1995); 1513-aromatic C=C stretch (Sun et al., 2005b); 1510-semi-circle stretch of para-substitute benzene rings (Budevska et al., 2002); 1550-protein (Budevska et al., 2002); 1502-aromatic skeletal vibration-softwood lignin (Pandey, 1999); 1505- aromatic skeletal vibration of benzene ring in lignin (Pandey, 1999)
1500-1450	-	1461	1467	1462-C-H lignin deformation (methyl and methylene) (Pandey, 1999).
1450-1400	1431	-	1429	1425- C-H in-plane lignin deformation with aromatic ring stretching (Pandey, 1999); 1430-CH_2 in-plane bending vibrations (Schulz and Baranska, 2007); 1430- C-H cellulose deformation (asymmetric) (Pandey, 1999); 1433-aromatic C=C stretch (Sun et al., 2005b).
1400-1350	1373	-	-	1370-weak C-O stretching (Yu, 2007; Wetzel et al., 1998); 1372-C-H cellulose deformation (asymmetric) (Pandey, 1999); 1380-C-H symmetric and asymmetric deformation (Sun et al., 2005b); 1382-C-O

				stretch (Xu et al., 2007); 1378-cellulose (microcrystalline) (Himmelsbach and Akin, 1998).
1350-1300	1319, 1338	-	-	1335-weak C-O stretching (Yu, 2007; Wetzel et al., 1998); 1336-C-H ring in-plane bending vibrations (Schulz and Baranska, 2007; 1336- O-H in-plane cellulose deformation (Pandey, 1999); 1318-CH_2 cellulose wagging (Pandey, 1999).
1300-1250	-	1251	-	1258- acetylated glucomannan (Himmelsbach and Akin, 1998); 1258- arabinoxylans (hemicellulose) (Xu et al., 2007).
1250-1200	1203	1213	-	1246 cellulosic material (Yu, 2007); 1246-weak C-O stretching (Wetzel et al., 1998); 1258- acetylated glucomannan (Himmelsbach and Akin, 1998); 1250-acetylated hemicelluloses (Budevska et al., 2002); 1201- O-H cellulose deformation (Pandey, 1999)
1200-1150	-	1166	1157	1160 glycosidic linkage (Robert et al., 2005); 1162-C-O-C ring vibrational stretching (Schulz and Baranska, 2007); 1165-arabinoxylans (hemicellulose) (Xu et al., 2007);
1100-1050	-	-	1054	1086- C-O of secondary alcohols (Pandey, 1999).
1050-1000	-	1050	-	1044-arabinoxylans (hemicellulose) (Xu et al., 2007); 1035-C-O, C = C and C-C-O vibrational stretching (Schulz and Baranska, 2007; 1045-C-OH bending Robert et al. 2005; Wetzel et al., 2003; 1025-non-structural CHO (Yu, 2007).

6.4.3 Radio Frequency-Alkaline Treatment on Lignin

It was observed that NaOH concentration is a major factor in the pretreatment process. The swelling initiated by NaOH creates pores in the biomass matrix (Nlewem and Thrash 2010; Fan et al. 1987; Sun and Cheng 2002), which helps enhance the reactivity of the biomass matrix to any externally added material such as enzyme (Iroba et al. 2013b). A similar finding was reported by Kumar et al. (2009). The lignin values obtained from the traditional wet chemistry

chemical composition analysis and through FTIR-PAS as shown in Table 6.4 depicts that in most cases, there is a decrease in the lignin in the pretreated samples as compared to the non-treated samples. This may be due to the unloosened lignified matrix in the non-treated sample which is tightly bound to the other constituents, unlike the RF alkaline pretreated samples with broken bonds which has been structurally separated, and disrupted. This disaggregation may be attributed to the interaction between the biomass and NaOH solution in the presence of the RF heating which is believed to be responsible for this solid loss (Iroba et al. 2013b). It also seems that the disruption and deconstruction of the lignified matrix is associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF radiation and the ions and molecules from the NaOH solution and the biomass (Iroba et al. 2013b). Lignin removal is an important part of the pretreatment process, because lignin can effectively inhibit/prevent the cellulase enzymes from hydrolysing the cellulose. This lignin reduction resulting from the alkaline pretreatment had also been reported by Xu et al. (2009) and Silverstein et al. (2007).

Lignin estimated by FTIR-PAS was higher than those obtained with NREL procedures. The laborious and time consuming traditional method that uses 72% H_2SO_4 seems to create a more stringent condition that may lead to altering and further degrading the native cell wall compositions (such as lignin and complex carbohydrates), structure, and possibly generating artifacts. FTIR-PAS is a rapid, direct, non-invasive, and non-destructive chemical analytical technique. This rapid method can detect molecular chemical characteristics of biological materials at high spatial resolutions without altering the inherent biomass structure such as the tissue (Yu 2005; Wetzel et al. 1998).

Table 6.4 Lignin composition of RF-alkaline pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Temperature (^{o}C)	Biomass: NaOH solution ratio	Lignin (%)		
		Wet chemistry	FTIR-PAS	Standard error
Non-treated	-	20.12	22.62	1.25
70	1:4	19.56	15.80	1.88
80	1:4	19.22	15.78	1.72
90	1:4	20.66	20.40	0.13
70	1:5	17.81	16.77	0.52
80	1:5	18.22	20.63	1.21
80^{W}	1:5	15.62	18.15	1.27
90	1:5	17.64	19.38	0.87
90^{W}	1:5	15.37	15.90	0.27
24	1:6	21.57	23.65	1.04
70	1:6	17.91	15.98	0.97
90	1:6	18.38	21.63	1.62
24	1:7	17.99	22.34	2.17
70	1:7	17.82	17.55	0.13
80	1:7	18.10	25.22	3.56
80^{W}	1:7	12.39	20.57	4.09
80^{DW}	1:7	19.90	27.74	3.92
80^{TW}	1:7	20.38	26.49	3.06
90	1:7	18.05	20.19	1.07
90^{W}	1:7	10.98	18.78	3.90
70	1:8	18.53	22.20	1.83
80	1:8	18.86	21.60	1.37
90	1:8	19.27	17.45	0.91

Standard error = standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; W = washed after pretreatment; DW = pretreated with distilled water; TW = pretreated with tap water.

The difference in the lignin values from the traditional approach and FTIR-PAS may also be attributed to the spectrum manipulations and the assumption that the reference lignin sample is 100% pure. The FTIR-PAS qualitative and quantitative analytical chemical information can be connected to the structural information within cellular dimension (Budevska 2002).

Equation 6.1 shows the predictive model for lignin generated from the training and verification analysis of the combined values from RF and SE using the methods described in section 6.3.8.

Lignin predictive model:

$$\%L = -33.92X_1 + 52.61X_2 + 32.16X_3 + 208.14X_4 + 98.46X_5 - 56.59X_6 + 17.33 + \varepsilon \quad (6.1)$$

%L = %lignin (dry matter basis), X_i = regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 6.1a), with the regressors representing the respective wavelengths as 1599 (X_1), 1511 (X_2), 1467 (X_3), 1429 (X4), 1157 (X_5), and 1054 cm^{-1} (X_6). 17.33 = intercept, and ε = error term/stochastic variable which describes the noise (errors that could emanate from the equipment, environment, or the experimenter).

Table 6.5 shows the R^2 and mean square error values of the various biomass components from the regression analysis. However, the R^2 values are too low; this might be due to the associated stochastic variables and the spectrum manipulations.

Table 6.5 R^2 and mean square error values from the regression analysis

Biomass composite	R^2 value	Mean square error
% Lignin	0.68	9.10
% Cellulose	0.34	13.86
% Hemicellulose	0.31	15.86

Washing the pretreated samples reduces the lignin. This may be due to loss of solid lignin during the washing process (Iroba et al. 2013b). This investigation shows that the concentration of NaOH solution and the ratio of biomass to the NaOH solution are the dominant contributing factors, while temperature plays a lesser role. The heat provided by the RF is needed to assist the alkaline solution in the deconstruction and disaggregation of lignocellulosic biomass matrix. It was also observed from this investigation that biomass can be alkaline pretreated even at room temperature if the required ratio of biomass and NaOH solution is applied.

6.4.4 Radio Frequency-Alkaline Treatment on Cellulose and Hemicellulose

The difference in the cellulose and hemicellulose values from the traditional approach and FTIR-PAS may be attributed to the spectrum manipulations and the assumption that the reference cellulose and hemicellulose samples are 100% pure. Equations 6.2-6.3 show the cellulose and hemicellulose predictive models generated from the training and verification analysis of the combined values from RF and SE using the methods described in section 6.3.8.

Table 6.6 Cellulose and hemicellulose compositions of RF-alkaline pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Temperature (^{o}C)	Biomass: NaOH solution ratio	Cellulose (%)			Hemicellulose (%)		
		Wet chemistry	FTIR-PAS	Standard error	Wet chemistry	FTIR-PAS	Standard error
Non-treated	-	42.51	50.37	3.93	29.98	23.82	3.08
70	1:4	22.25	28.65	3.20	23	18.10	2.45
80	1:4	22.37	27.69	2.66	22.14	28.47	3.16
90	1:4	26.93	26.09	0.42	26.24	20.73	2.75
70	1:5	24.21	4.98	9.61	21.63	30.37	4.37
80	1:5	21.07	29.42	4.18	21.38	24.56	1.59
80^{W}	1:5	27.69	26.55	0.57	21.6	15.81	2.90
90	1:5	24.65	37.03	6.19	21.05	8.44	6.31
90^{W}	1:5	33.44	34.50	0.53	26.08	22.58	1.75
24	1:6	30.93	14.95	7.99	29.12	21.00	4.06
70	1:6	28.25	29.02	0.39	23.18	22.88	0.15
90	1:6	30.37	20.85	4.76	22.36	39.31	8.47
24	1:7	25.78	27.08	0.65	23.4	26.21	1.40
70	1:7	27.39	11.74	7.82	24.27	28.81	2.27
80	1:7	26.08	17.83	4.12	26.75	10.70	8.02
80^{W}	1:7	38.20	19.86	9.17	27.72	8.48	9.62
80^{DW}	1:7	21.88	2.06	9.91	19.12	5.76	6.68
80^{TW}	1:7	24.98	6.09	9.44	21.04	9.97	5.54
90	1:7	21.33	23.74	1.20	22.27	9.55	6.36
90^{W}	1:7	33.31	12.98	10.17	20.69	9.71	5.49
70	1:8	26.73	27.91	0.59	26.76	22.69	2.03
80	1:8	18.44	42.08	11.82	18.32	31.06	6.37
90	1:8	22.68	21.66	0.51	19	25.64	3.32

Standard error = standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; W = washed after pretreatment; DW = pretreated with distilled water; TW = pretreated with tap water.

Cellulose predictive model:

$$\%C = 28.63X_1 + 48.60X_2 + 35.83X_3 - 51.71X_4 - 29.24X_5 + 37.16 + \varepsilon \quad (6.2)$$

Where %C = %cellulose value, X_i = regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 6.1b), with the regressors representing the respective wavelengths as 1431 (X_1), 1373 (X_2), 1338 (X_3), 1319 (X_4), and 1203 cm^{-1} (X_5), 37.16 = intercept, and ε = error term/stochastic variable.

Hemicellulose predictive model:

$$\%H = -14.25X_1 - 90.42X_2 + 34.14X_3 - 39.18X_4 + 71.17X_5 + 122.90X_6 + 30.42 + \varepsilon \quad (6.3)$$

%H = %hemicellulose wet chemistry value, X_i = regressors/explanatory variables (normalized data based on the respective characteristic peaks, as shown in Figure 6.1c), with the regressors representing the respective wavelengths as 1606 (X_1), 1461 (X_2), 1251 (X_3), 1213 (X_4), 1166 (X_5), and 1050 cm^{-1} (X_6), 30.42 = intercept, and ε = error term/stochastic variable.

Ramesh and Singh (1993) reported that barley straw theoretically contains about 40% cellulose, 20% hemicellulose, and 15% lignin. Marsden and Gray (1985) also reported that barley straw theoretically contains about 44% cellulose, 27% hemicellulose, and 7% lignin. These values are comparable with the values obtained from the non-treated sample in this investigation. It should be noted that the variance in the chemical composition between the reported theoretical values and the values obtained from this investigation may be attributed to differences in locations where the crop was grown, weather conditions, the barley variety grown, and different methods of analysis.

6.4.5 Steam Explosion Treatment on Lignin

The efficacy of FTIR-PAS techniques for studying changes in plant cell wall composition following steam explosion pretreatment has been evaluated. Table 6.7 shows that the SE pretreated samples have higher lignin content as compared to the non-treated. This may be due to the carbonization of the sample resulting from the direct contact of biomass with the walls of the reactor during the steam explosion pretreatment.

Table 6.7 Lignin composition of SE pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Temperature (^{o}C)	Moisture content (%)*	Retention time (min)	Lignin (%)		
			Wet chemistry	FTIR-PAS	Standard error
Non-Treated	-	-	20.12	22.62	1.25
140	8	5	23.79	26.25	1.23
160	8	5	22.72	31.46	4.37
180	8	5	40.58	21.41	9.58
140	30	5	22.05	28.23	3.09
160	30	5	21.69	24.53	1.42
180	30	5	33.01	25.99	3.51
140	50	5	21.18	25.41	2.11
160	50	5	23.31	33.21	4.95
180	50	5	25.04	32.60	3.78
140	8	10	21.56	31.63	5.04
160	8	10	21.66	25.13	1.73
180	8	10	32.75	33.45	0.35
140	30	10	21.25	29.37	4.06
160	30	10	20.9	27.47	3.28
180	30	10	37.31	29.21	4.05
140	50	10	21	23.54	1.27
180	50	10	31.82	31.16	0.33

Standard error = standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; * = % mass fraction of water.

Hemicellulose degrades easily and some volatile organic compounds vaporize as volatile components, while cellulose behaves as a fixed carbon (solid combustible residue). This may account for the increase in the lignin content. Lam et al. (2011) investigated the steam explosion of Douglas fir (*Pseudotsuga menziesii*) at a reaction temperature of 200-220^{o}C and a retention time of 5-10 min. These researchers reported that there was increase in lignin content from 30 to 43% attributed to the thermal degradation of hemicellulose during the steam explosion treatment. Chen and Kuo (2011) reported that cellulose and lignin are both locked in biomass from the mild carbonization process. This indicates that the degraded cellulose may appear as residue resulting to the increase in the lignin content.

The interaction between moisture content and temperature and also between retention time and moisture content had a statistically significant effect ($P<0.01$) on the lignin. The interaction among the three variables (moisture content, temperature, and retention time) also had a significant effect on the lignin. The difference in the lignin values obtained using the FTIR-PAS and the traditional approach may be attributed to the spectrum manipulations and the assumption that the reference lignin sample is 100% pure.

6.4.6 Steam Explosion Treatment on Cellulose and Hemicellulose

From Table 6.8, in comparison with the non-treated biomass, it is evident that retention time, moisture content, and temperature had a significant effect on the cellulose and hemicellulose content. The decrease in the sugars content increased at higher retention time and temperature. Wang et al. (2009) also reported that the retention time and temperature are the process parameters required for the optimization of steam explosion process. In this present study, less degradation of the simple sugars was observed at higher moisture content. High feedstock moisture content acts as acid catalyst to hydrolyze biomass during steam explosion. However, the direct contact of biomass with the walls of the reactor will limit and affect the extent of the hydrolysis. Therefore, a combination of carbonization and acid catalyzed hydrolysis occurred which ultimately led to the degradation of the simple sugars and increase in the lignin. The obtained results from the wet chemistry demonstrated that the hemicellulose contained in the biomass was highly degraded (79% to 89%) compared to cellulose (58% to 77%) as reported in chapter 4. The high degradation of hemicellulose was due to its amorphous nature, which degrades easily and evaporates as volatile components during the carbonization process. Presumably, the crystallinity of cellulose was responsible for the less degradation of this component. These degradations can be explained by considering the monomers of hemicellulose and cellulose which consist primarily of sugars. Degradation of cellulose and hemicellulose during steam explosion/thermal pretreatment of biomass has been reported by Toussaint et al. (1991), Excoffier et al. (1988), Ferro et al. (2004), Yan et al. (2009) Chen and Kuo (2010) and Heitz et al. (1991). It was reported that hemicellulose is very reactive and was nearly completely removed at 200^{o}C, while both cellulose and lignin can be dissolved partially at higher temperatures. Shaw (2008) performed steam explosion on poplar and wheat straw at 200-205^{o}C,

steam pressure of 1.66-1.73 MPa for 4-4.5 min. This author reported a decrease in cellulose and hemicellulose content with an increase in the lignin content after the steam explosion treatment of both biomass samples. Chen and Kuo (2011), Yang et al. (2007), and Khezami et al. (2007) studied the effects of thermal process on biomass. These researchers showed that thermal pretreatment removes moisture and light volatiles from biomass. Bergman et al. (2005) and Lipinsky et al. (2002) reported that during the torrefaction process, biomass was partly decomposed giving off various condensable and non-condensable gases, with a carbon-rich solid as a final product. Mohammad and Karimi (2008) reported a corresponding decrease in total sugar recovery with increasing temperature during steam explosion.

Furthermore, the difference between the cellulose and hemicellulose values estimated using the FTIR-PAS and the measured values using the traditional approach might also be associated with the spectrum manipulations and the assumption that the reference cellulose and hemicellulose samples are 100% pure. The main advantage of this correlation is that based on collection of FTIR-PAS spectra, it provides a rapid, easy, economical, non-destructive, and non-laborious estimation of the chemical composition of biomass. This may be of particular interest in the contexts where more sophisticated and expensive equipments for experimental measurement of biomass chemical compositions are not always available. However, care must be taken in selecting the steam explosion pretreatment conditions in order to prevent excessive degradation of the chemical properties of the complex carbohydrates, since the yields of hemicelluloses and cellulose were dependent on the pretreatment conditions of the steam explosion.

Table 6.8 Cellulose and hemicellulose compositions of SE pretreated and non-treated biomass grind obtained using the traditional wet chemistry and FTIR-PAS methods

Tempt. (°C)	M.C. (%)	R.T. (min.)	Cellulose (%)			Hemicellulose (%)		
			Wet chemistry	FTIR-PAS	Standard error	Wet chemistry	FTIR-PAS	Standard error
Non-treated	-	-	42.51	50.37	3.93	29.98	23.82	3.08
140	8	5	11.19	20.53	4.67	3.42	12.39	4.48
160	8	5	11.17	17.93	3.38	3.29	13.10	4.90
180	8	5	9.58	18.54	4.48	3.74	6.60	1.43
140	30	5	14.82	19.87	2.53	3.8	17.79	7.00
160	30	5	14.63	17.53	1.45	4.13	12.54	4.21
180	30	5	12.07	17.30	2.62	3.88	11.44	3.78
140	50	5	15.81	22.06	3.12	6.58	15.95	4.69
160	50	5	14.51	19.74	2.62	5.11	15.18	5.04
180	50	5	15.77	20.24	2.23	5.23	14.02	4.40
140	8	10	10.02	16.91	3.45	3.9	16.01	6.05
160	8	10	9.11	19.49	5.19	4.47	9.44	2.49
180	8	10	8.74	16.08	3.67	3.23	12.03	4.40
140	30	10	15.38	15.66	0.14	5.03	14.91	4.94
160	30	10	13.57	16.95	1.69	6.59	6.50	0.05
180	30	10	12.7	16.24	1.77	4.54	12.47	3.96
140	50	10	16.02	17.51	0.74	6.33	14.01	3.84
180	50	10	12.35	15.95	1.80	4.69	10.70	3.01

Standard error = standard deviation between the wet chemistry and FTIR-PAS values divided by the square root of 2; Tempt. = temperature; M.C. = moisture content; R.T. = retention time.

6.5 Conclusion

FTIR-PAS was used in light of the need for rapid analysis of biomass materials and wood-based materials at large. The samples were initially pretreated using RF-alkaline and steam explosion techniques and analyzed gravimetrically using the traditional approach to elucidate compositional information. Thereafter, the effect of the pretreatment conditions on barley straw grind was further analyzed based on their FTIR-PAS spectra. The FTIR-PAS technique has advantage because it is a quick, easier, and non-destructive method. The structure of the biomass is maintained when spectra are measured directly from the bulk of grind biomass surface. This implies that the PA infrared spectra can be used for biofuel feedstock identification and analysis of the chemical composition of biomass before it is processed.

Chapter 7

7. Producing Durable Pellets from Barley Straw Subjected to Radio Frequency-Alkaline and Steam Explosion Pretreatments

A similar version of this chapter has been published in the International Journal of Agricultural and Biological Engineering:

Iroba, K.L., L.G. Tabil, S. Sokhansanj and V. Meda. 2014. Producing durable pellets from barley straw subjected to radio frequency-alkaline and steam explosion pretreatments. *International Journal of Agricultural and Biological Eng*ineering 7(3): 68-82.

In the course of this Ph.D. research, the preliminary outcome of this investigation was presented and published in an international conference:

- Iroba, K.L., L.G. Tabil, T. Ca*nam and T. Dumonceaux. 2012. Densification of radio freq*uency pretreated lignocellulosic biomass barley straw. ASABE Annual International Meeting, Dallas, TX, Paper No. 121337630, July 29-August 1.

Contributions of Ph.D. Candidate

The quality parameter changes of manufactured pellets from radio frequency-alkaline and steam explosion pretreated, and non-treated lignocellulosic biomass barley straw were assessed. These quality characteristics (density, tensile strength, durability, and dimensional stability) can be used by the biofuel industry (pellets manufacturers) to perform relative comparison of quality of pellets produced from any kind of agricultural biomass. These quality parameters were used as tools to evaluate the effectiveness of the material and operating variables of the pretreatment process. Experimental design, data analysis, and writing of journal manuscript were performed by Kingsley Iroba, while Dr. Lope Tabil, Dr. Shabab Sokhansanj, and Venkatesh Meda provided

editorial input. Riley Woods, a summer undergraduate student in Chemical and Biological Engineering Department worked on this project and performed most of the densification of radio frequency pretreated samples under my supervision.

Contributions of this Paper to Overall Study

Knowledge Gap: Perusal of literature on densification vs pretreatment showed that there is a knowledge gap related to the response of quality parameters of lignocellulosic biomass to radio frequency alkaline and steam explosion pretreatment at low severity factor. The effects of ratio of biomass to alkaline solution using radio frequency technique on manufactured pellets has not been studied or reported. Additionally, the response of quality parameters of biomass pellets resulting from steam explosion pretreatment at low severity factor has also not be investigated in the past. Therefore, the objectives of this study was to investigate the effects of radio frequency alkaline and steam explosion pretreatments on the physical characteristics (density, tensile strength, durability, and dimensional stability) of pelletized barley straw, which could be used as feedstock for the production of bio-energy via thermochemical process.

Justification: Agricultural crop residues are considered as good feedstocks for biofuel applications, due to the low nutritional value when used as feed for animals (Tavakoli et al. 2009). Saskatchewan Ministry of Agriculture reported that in 2013, Canada produced about 69.6 million tonnes (mt) of wheat, canola, barley, and oat, with Saskatchewan production at 33 mt. Barley production was at 3.4 mt and 10.2 mt for Saskatchewan and Canada, respectively. Montane et al. (1998) reported that an average ratio of 1.3 kg of straw per kg of wheat grain harvested is obtained for most common varieties of wheat. This translates to total production in 2013 to about 42.8 mt and 90.5 mt of straws for Saskatchewan and Canada, respectively.

Tabil et al. (2011) reported that biomass (dry plant matter) has energy content in the range of 16-20 MJ/kg. Biomass as an energy source and as an important feedstock for the biorefinery industries does not provide economical, efficient, and easy handling, transportation, and storage characteristics as a result of the large volume requirements (Tabil 1996; Tabil et al. 2011; Mani et al. 2006b; Adapa et al 2009a). To decrease the associated costs of storage, production, and transportation of biofuel, it is considered of great value to densify the biomass, while increasing

its higher heating value per unit volume (Tabil et al. 2011; Kaliyan and Morey 2006). The bulk density of loose dry biomass cereal straw (barley, wheat) in their natural form can be as low as 40 kg/m^3, 40-80 kg/m^3 for corn stover, and 250 kg/m^3 for some wood residues (Tabil et al. 2011; Sokhansanj et al. 2005). Lignin has binding agent characteristics that holds plant together and gives it rigidity (Fan et al. 2006a; Lin and Tanaka 2006). Studies have showed that modification of the structural distribution and arrangement of cellulose-hemicellulose-lignin matrix can effectively improve the natural binding characteristics of the lignocellulosic straw and stover biomass (Sokhansanj et al. 2005). This implies that the natural binder present in lignocellulosic biomass needs to be harnessed through pretreatment process. Thereafter, the pretreated biomass can be converted and densified to high-density and high-value solid format like pellets to improve the convenience and accessibility of biomass.

7.1 Abstract

Pelletization, a form of densification, increases bulk density and improves the convenience and accessibility of biomass feedstock due to the uniform shape and size. Pretreatment of biomass enhances the breakdown and accessibility of the cross-linking lignin, which acts as a binding agent. In this study, pelletization of radio frequency-alkaline and steam explosion pretreated barley straw was performed. Three levels of temperature (70^{o}C, 80^{o}C, and 90^{o}C), five levels of the mass ratio of biomass to NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8), 1 h soaking time, biomass screen size of 1.6 mm, 1% NaOH concentration, and 20 min residence time in the radio frequency chamber; were used for the radio frequency-alkaline pretreatment. Three levels of steam temperature (140^{o}C, 160^{o}C, and 180^{o}C), three levels of moisture content of 8, 30, and 50% (mass fraction of total mass), and 5 and 10 min exposure to steam were tested for the steam explosion pretreatment. The effects of both pretreatment methods were evaluated by pelletizing the pretreated and non-pretreated barley straw samples in a single pelleting unit. The pellet density, tensile strength, durability, dimensional stability, and color of the pellets were determined. Radio frequency-alkaline pretreatment with the use of 1% NaOH solution and a ratio of biomass:NaOH solution of 1:8 has significant effect ($P<0.05$) on the breakdown of the lignified matrix, resulting in pellets with superior physical characteristics. The steam exploded

samples pretreated at higher temperatures (180ºC) and retention time of 10 min resulted into pellets with good physical qualities.

7.2 Introduction

The reliance on fossil fuel (coal and petroleum) of the energy infrastructure in most parts of the world causes serious environmental problems. In recent times, there has been a high level of consciousness and awareness to reduce the dependence on fossil fuels. There is tremendous interest and emphasis towards sustainable and environmentally friendly sources of alternative fuels (Fiona et al. 2007). As part of the strategies to lessen the severity of the above mentioned effect; decrease the carbon footprint and also improve sustainability of energy supply, lignocellulosic biomass is one of the substitute that is readily available, renewable, and carbon sink source of energy (Demirbas et al. 2009), with annual production of approximately 200 billion tonnes worldwide (Zhang 2008). Lignocellulosic biomass straws are main portions of crop residues which are considered as important feedstocks for biofuel applications, because they have low nutritional value when used as feed for animals (Tavakoli et al. 2009).

Lignocellulosic biomass is a complex formation of cellulose, hemicellulose, and lignin. The lignin (which is normally deposited at maturity) acts as an external cross-linked aromatic polymer based on phenylpropanoid units binding hemicellulose and cellulose with cellulose positioned at the inner core of the structure (Fan et al. 2006b). Modification of the structural arrangement of cellulose-hemicellulose-lignin matrix during the pretreatment process can enhance the binding characteristics of lignocellulosic straw biomass, without necessarily using any expensive artificial binder during pelletization (Iroba and Tabil 2013). Radio frequency (RF) heating has been applied as a treatment method on lignocellulosic biomass (Iroba et al. 2013), to prevent the growth of fungus in wood materials in an industrial 40 kW dielectric oven at temperatures range of 60°C to 70°C for 2 min (Tubajika et al. 2007). RF has been successfully used for leather drying (Balakrishnan et al. 2004), thermal therapy (Brausi et al. 2004), and in other research fields such as food processing (blanching, tempering, pasteurisation, sterilisation) and medicine (Piyasena et al. 2003). Recently, Izadifar et al. (2009) demonstrated that RF can be used for the extraction of podophyllotoxin from rhizomes of *Podophyllum peltatum*. The use of

steam explosion as a pretreatment method on the lignocellulosic biomass have been extensively studied by Toussaint et al. (1991); Mosier et al. (2005); Tanahashi et al. (1988); Chornet and Overend (1988); Heitz et al. (1991); Chundawat et al. (2006); Chum et al. (1990); Kaar et al. (1998).

Biomass can either be used directly for combustion and co-firing for home heating, providing process heat for industrial facilities, generation of electricity, or application as liquid and gas fuels in the form of bioethanol or biogas, as well as a source of variety of bioproducts (Demirbas et al. 2009). Production of energy from biomass will to a large extent complement energy from fossil fuel even if it cannot completely replace it in this century. Loose plant-based biomass has low bulk density depending on the plant species, particle density and particle size (Sokhansanj and Turhollow 2004). In their natural form, biomass crops are bulky and dispersed, hence they are difficult to handle and utilize as a fuel (Tabil 1996). The bulk density of loose dry biomass cereal straw (wheat, barley) can be as low as 40 kg/m^3, 40-80 kg/m^3 for corn stover, and 250 kg/m^3 for some wood residues (Tabil et al. 2011; Sokhansanj et al. 2005). Biomass has energy content in the range of 16-20 MJ/kg for dry plant matter (Tabil et al. 2011). Biomass as an energy source and as a feedstock for biorefineries does not present easy, economically feasible and efficient transportation, handling and storage characteristics as a result of large volume requirements (Tabil 1996; Tabil et al. 2011; Mani et al. 2006b; Adapa et al 2009a). To reduce the production, transportation, and storage costs of biofuel, it is importance to densify the biomass, while increasing its heating value per unit volume (Tabil et al. 2011; Kaliyan and Morey 2006). Densification, particularly pelletization, is an important strategy for the biomass market, because it improves the convenience and accessibility of biomass due to the uniform shape and size, clean, stable fuel. A pelletized biomass can be easily adopted into the "direct-combustion or co-firing with coal, gasification, pyrolysis, and biomass-based" conversion reactors (Granada et al 2002; Kaliyan and Morey 2009b). Pelletization increases flowability of biomass products, decreases the porosity, spillage and wind loss, and improves its utilization performance (Briggs et al. 1999; Obernberger and Thek 2004; Adapa et al. 2009a; Adapa et al 2009b). In spite of the complications of handling, the direct combustion of loose biomass in traditional combustion chambers "is associated with very low thermal efficiency. Conversion efficiencies may be as low

as 40% with widespread air pollution in the form of very fine" particulates matters with increased risk of fire and explosions (Tabil et al. 2011; Gover and Mishra 1996). Development of a large-scale biomass unit, requires the conversion of biomass into high-density and high-value solid format like pellets. Manufacturing good quality pellets, briquettes and cubes "is largely thought as an art rather than a science by many operators" (Tabil et al. 2011; Briggs et al. 1999). Cost is a challenge in biomass densification because of the high energy consumption. Low production costs, high quality pellets and briquettes together with safe handling of biomass will make biomass competitive with fossil fuels.

The objective of this study was to investigate the effects of radio frequency-alkaline and steam explosion pretreatments on the physical characteristics (density, tensile strength, durability, and dimensional stability) of densified barley straw, which could be used as feedstock for the production of bio-energy via thermochemical process.

7.3 Material and Methods

7.3.1 Sample Collection and Preparation

Barley straw of the 'Xena' variety was obtained in October, 2009 from RAW Ag Ventures Limited, (Maymont, SK) and was grown at 52.667297º N and 107.794311º W. The straw was ground using a hammer mill (Glen Mills Inc., Maywood, NJ) with screen size of 1.6 mm to increase the surface area of the biomass. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to provide flow of the biomass in and out of the hammer mill, to control dust and collect the ground biomass during operation. The initial moisture content of the straw was 8.09% (mass fraction of total mass). The moisture content was measured based on ASABE standard method, ASAE S358.2 DEC1988 (2008). This test was performed in replicates of three. For details of the measurement, calculations, and results of the physical characteristics of the barley straw grind, see Iroba et al. (2013).

7.3.2 Radio Frequency Pretreatment

The pretreatment was performed using radio frequency (RF) machine (1.5 kW and 27.12 MHz laboratory dryer, Strayfield, Theale, Reading). To understand the interactions between the process and material variables, the study was based on a factorial experiment in a completely randomized design. The variables shown in Table 7.1 and a blown glass reactor (4.25 L volume) with the dimensions shown in Figure 7.1 were employed.

Table 7.1 Material and operating variables during radio frequency alkaline pretreatment of ground barley straw using a blown glass reactor

Variables	Levels
Hammer screen size	1.6 mm
NaOH solution concentration	1% w/v
Biomass: NaOH solution ratio	1:4 - 110 g biomass and 440 g NaOH solution 1:5 - 110 g biomass and 550 g NaOH solution 1:6 - 100 g biomass and 600 g NaOH solution 1:7 - 100 g biomass and 700 g NaOH solution 1:8 - 90 g biomass and 720 g NaOH solution
Soaking time	1 h
Temperature	70, 80, and 90 ^{o}C
Residence time	20 min

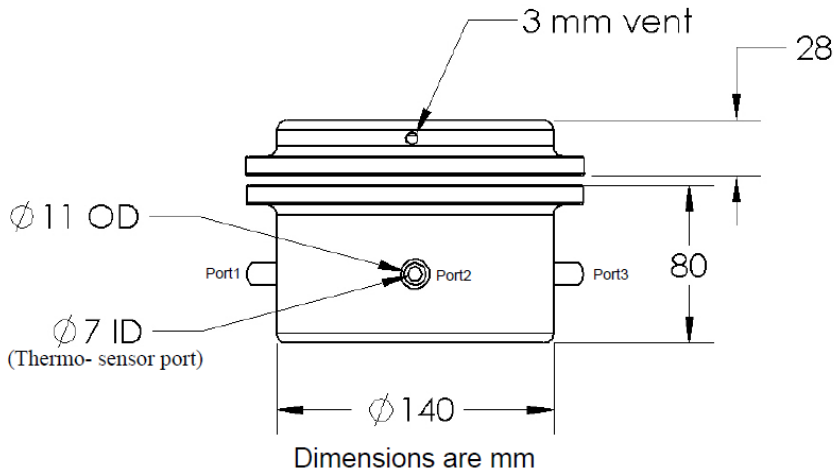


Figure 7.1 Schematic diagram of blown glass reactor used for radio frequency pretreatment of samples: ID – internal diameter, OD – outer diameter. The vent is use for pressure release, to avoid pressure build up within the reactor.

The ground barley straw samples and the 1% w/v NaOH solution were mixed thoroughly in the reactor based on the ratio indicated in Table 7.1. Selective heating (due to presence of aqueous solution which results in excitation to different levels at various locations within the reactor) can also be minimised by adequate mixing. For details of the radio frequency-assisted alkaline pretreatment see Iroba et al. (2013).

7.3.2.1 Washing of the RF-Alkaline Pretreated Samples

Four treatment combinations were randomly selected for washing to remove the NaOH solution in the RF alkaline pretreated samples. After heating the mixture of biomass-NaOH solution in the RF machine, it was placed in a vacuum filter consisting of a perforated porcelain funnel 200 mL with Whatman filter paper (size 40). Tap water (approximately 1 L) was repeatedly used for the washing until the pH reached around 7.0. The RF alkaline pretreated washed samples were dried to 10% moisture content (mass fraction of total mass) using a forced-air convection dryer (Shaw et al. 2007) set at 40^{o}C.

7.3.3 Steam Explosion Pretreatment

Ground barley straw samples were re-conditioned to the desired moisture content by spraying a calculated amount of water on the samples, mixed thoroughly and kept in a conditioned environment (~6^{o}C) for 48 h before the pretreatment process. Three process parameters were studied for the steam explosion (SE) treatment. These variables (temperature, moisture content, and retention time) were investigated to determine the optimum condition for steam explosion pretreatment of ground barley straw, to enhance formation of pellet with good quality characteristics. The experimental design was based on completely randomized approach, which resulted in eighteen treatment combinations. Table 7.2 shows the levels of these independent variables:

Table 7.2 Independent variables with corresponding levels used for the steam explosion pretreatment of ground barley straw

Variables	Levels
Temperature (^{o}C)	140, 160, 180
Moisture content (% mass fraction of total mass)	8, 30, 50
Retention time (min)	5, 10

A temperature range of 140-180^{o}C was selected to pretreat the biomass within the softening temperature range for lignin. It was reported that the softening temperature of lignin is in the range of 130-190^{o}C, and the thermal stability of probable linkages between lignin and polysaccharides is compromised at temperatures above 200^{o}C (Fengel and Wegener 1984). Thermal degradation of lignin starts at 270-280^{o}C (Fengel and Wegener 1984).

7.3.3.1 Steam Explosion Process Description and Operation Procedures

The steam explosion pretreatment set up used for this investigation is located at the Clean Energy Research Center, University of British of Columbia. Figure 7.2 shows the process flow sheet of the closed system steam explosion batch unit:

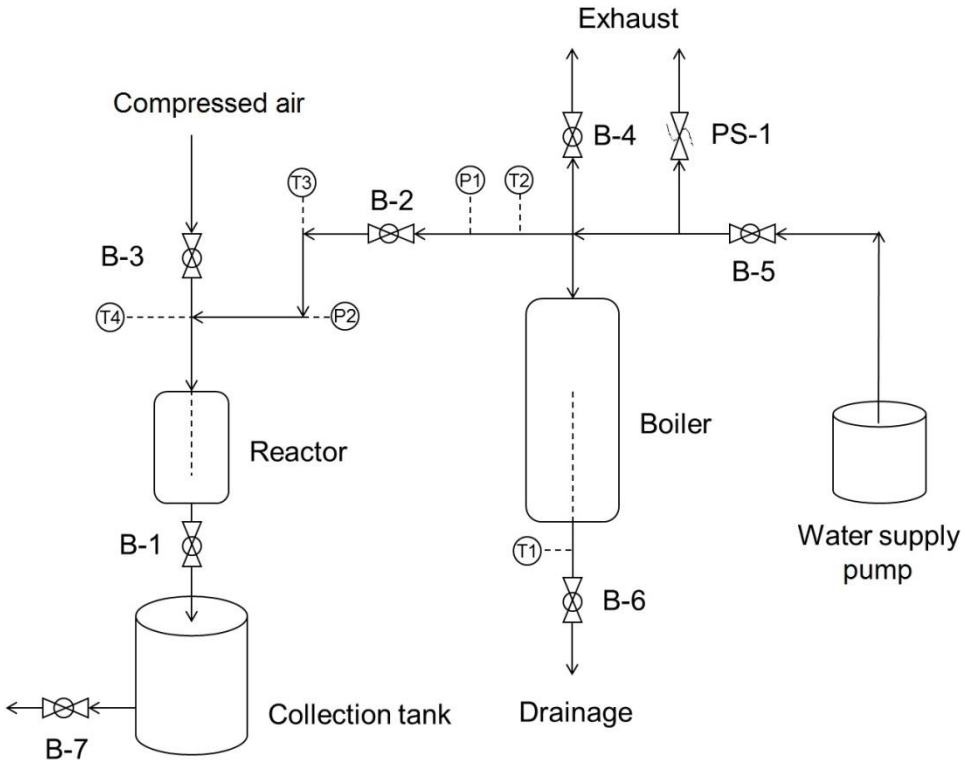


Figure 7.2 Process flow diagram of the closed system steam explosion unit (B: Ball valve, PS: Pressure relief valve, P: Digital pressure transducer, T: Thermocouple) (Lam 2011).

Biomass of about 28-30 g was loaded through the small opening (about 10 mm) at the top of the reactor. The unit consisted of a 2 L steam generator, which generates saturated steam, and a 1 L reactor for steaming lignocellulosic biomass feedstock. A 3 zone tubular furnace (Lindberg/Blue

M, STF55666C, Thermo Fisher Scientific Inc., Waltham, MA) was used for the generation of the steam heat. The 1 L reactor was equipped with a 12.7 mm diameter ball valve, controlled by an electrical actuator for sudden discharge of the treated biomass into the collection vessel at ambient pressure. The service and maintenance heat of the reactor and the steamline were supplied by a temperature controller. The details and functions of valves in the experimental setup unit are summarized elsewhere (Lam 2011). Temperatures and pressures were measured by 1.6 mm diameter K-type thermocouples (Omega, Stamford, CT) and digital pressure transducers (Omega, Stamford, CT), respectively. Data were collected by LabView 8.2 software (National Instruments, Austin, TX). The collected steam exploded solid biomass product was weighed to determine the mass loss or gain and kept in sealed Ziploc^{TM} containers at 6^{o}C for further analysis. Each test was replicated twice. For details of the steam explosion pretreatment see Iroba et al. (2014a).

7.3.4 Densification of RF-Assisted Alkaline and Steam Exploded Pretreated and Non-Treated Samples

To evaluate the efficiency of the RF-assisted alkaline and steam explosion pretreatment processes, densification of the pretreated and non-treated samples was performed. The samples were densified using a single pelleting unit mounted in an Instron tester (Model No. 3366, Instron Corp., Norwood, MA). This pelleting unit includes a steel cylindrical die with a plunger/piston connected to the upper moving crosshead which provides the load necessary to compress the biomass materials. The die is surrounded with a heating element to provide the required heat for the process. About 500-700 mg of the biomass feedstock was loaded into the die cylinder. The pre-set load (4 kN) and the required temperature (95±0.3^{o}C) were adjusted to compress the charge material. Once the pre-set load was realized, the plunger was stopped and held in position for 60 s retention time to avoid spring-back effect of biomass (Mani et al. 2006b), thereafter, the pellet was ejected. The Instron was typically set to lower the plunger (and compress the biomass) at a rate of 50 mm/min. The single pelleting unit was connected to a computer which recorded the time and force-displacement data. Eighteen replicates were produced for each sample. The pellets were stored at room condition (to simulate the real condition during storage or transportation) for further analysis.

7.3.4.1 Durability Test

The durability of the pellets was measured after two weeks of storage, by dropping the pellets from a height of 1.85 m (Al-Widyan and Al-Jalil 2001). This represents the agitation, rotating, shear, impact, and tumbling during transportation, storage and handling. The percentage durability was calculated by dividing the remaining piece by the original mass and multiplied by 100. Ten replicates were made for each sample.

7.3.4.2 Tensile Strength

Diametral compression test was applied to assessed the tensile strength of the biomass pellets (Tabil 1996) using the Instron tester. Pellets were cut diametrically using a scalpel into specimens with thickness of about 2.50 mm. The specimen was positioned on its edge on the lower padded plate and compressed with 1000 N load cell by the upper plunger (also padded) at a crosshead speed of 1 mm/min until failure occurred. Fractures that caused the specimens to break or crack in two halves along the loading axis were accepted and other fracture types were discarded (Fell and Newton 1968; Fell and Newton 1970). Upon failure, the fracture force was noted, while the tensile strength of tablets was evaluated using equation 7.1 below. Ten replicates were made for each sample.

$$\delta_x = \frac{2F}{\pi dl} \tag{7.1}$$

Where δ_x is tensile (horizontal) stress (Pa), F the load at fracture (N), d the specimen diameter (6.67 mm), and l the specimen thickness (mm).

7.3.4.3 Pellet Density and Dimensional Stability

The mass, length, and diameter of the pellets were measured immediately after pelleting and after 14 d of storage. These parameters were used to calculate the density. The change in density after two weeks of storage was used to evaluate the dimensional stability of the pellets.

7.3.5 Color Measurement

The color difference between the pretreated and non-treated was measured and compared using the HunterLab Color Analyzer (Hunter Associates Laboratory Inc, Reston, VA). The color was characterized using a three-dimensional color parameters (CIE L*, a* and b*). The scale L* ranges from 0 for black to +100 for white. The a* and b* have no specific numerical limits; Positive a* is red; Negative a* is green; Positive b* is yellow; Negative b* is blue. The total color difference, ΔE*, was also computed using equation 7.2 (Hunter Associates Laboratory Inc, Reston, VA). This single value takes into account the differences between the L*, a*, and b* of the pretreated and the control (non-treated) material. Three replicates were performed for each sample.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (7.2)$$

$\Delta L^* = L^*_{Treated} - L^*_{NT}$; $\Delta a^* = a^*_{Treated} - a^*_{NT}$; $\Delta b^* = b_{*Treated} - b^*_{NT}$. These parameters ΔL*, Δa*, and Δb* indicate how much the non-treated (NT) and the pretreated differ from one another in L*, a*, and b*.

7.3.6 Statistical Analysis

7.3.6.1 Analysis of Variance (ANOVA)

Linear regression was performed on the collected data to investigate the main effects of the independent variables on the dependent variables at 95% confidence level using IBM SPSS Statistics (Superior Performing Statistical Software, version 20 for Windows, 2012; IBM, Armonk, New York, NY). Further analysis was also performed to evaluate the effects of the interaction among the independent variables on the response variables.

7.3.6.2 Tukey's Statistical Comparison of Means on Pellet Properties

ANOVA shows if the independent variables have significant effect or not on the dependent variables. However, the ANOVA does not indicate where the differences lie. For this reason, there is a need to carry out one more statistical analysis to elucidate the mean differences. Statistical comparison of means using the Tukey's test method was performed on density, durability, tensile strength, and fracture load to evaluate the mean differences among the variables.

7.4 Results and Discussion

7.4.1 Force-Time Densification Profile

Figure 7.3 depicts the densification profile (force-time graph) of a ground barley straw sample. A-E represents the different stages of the densification process. The starting stage of the process is represented by A which is prior to application of pressure on the biomass grind. The three main stages involved during the densification process upon the application of pressure as summarized by Tabil (1996) are represented by B-D. The particle rearrangement and elastic and plastic deformation are represented by B-C. Particle rearrangement involves the rearrangement of the particles and breaking down the initial unstable packing arrangements, which is often called 'arches' or 'bridges'; this stage happens at low pressures resulting to a closer packing. The initial physical qualities of the original particles are maintained. Energy is expended as a result of particle-wall friction. The elastic and plastic deformation happens at higher pressures and involves two stages that correspond to elastic and plastic deformation. Due to the higher pressures, the particles are force to flow into the existing void spaces, resulting in porosity decrease which increases the contact area between the particles. At this point, if the pressure is released, the particles can recover their former state (elastic deformation). Continuous application of the pressure leads to particle fracture into fragments, followed by rearrangement of the fragments (plastic deformation). This stage progresses until the compact density is close to the solid density of the material. The third stage where the predefined retention time is applied involves the mechanical interlocking of particles and local melting of some constituents (Tabil 1996), which may result from the two earlier stages above. This stage is represented by C-D. Local melting of the materials occurs if the melting points of the constituents (such as lignin) are reached, causing binding. If this stage is not reached, it can lead to disintegration of the pellets/briquettes (Tabil 1996). DE represents the instantaneous time when the ejection of the produced pellet was initiated. The drop from D to E is gradual when the produced pellet is expelled from the die.

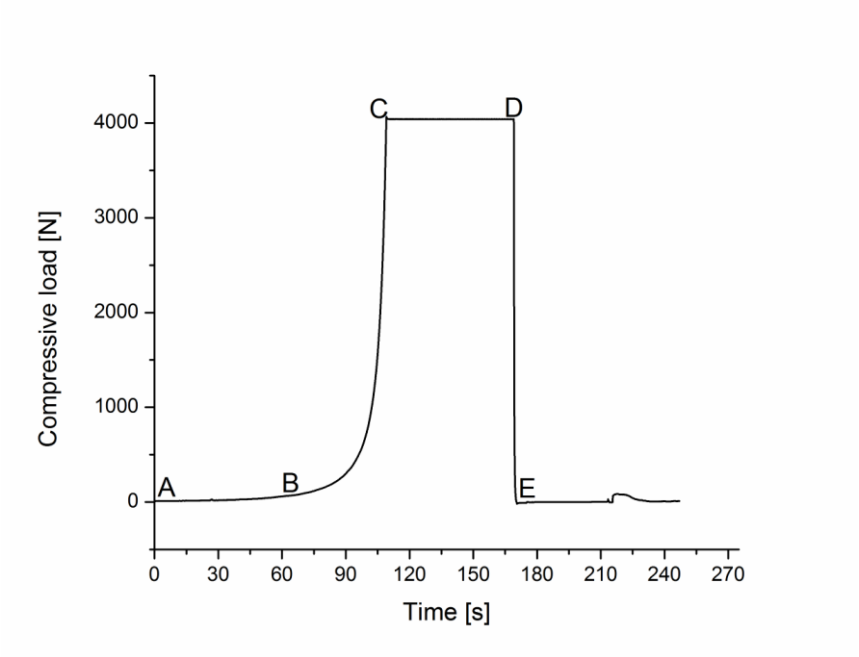


Figure 7.3 Typical force-time densification profile of ground barley straw sample.

7.4.2. Durability Rating, Tensile Strength, and Fracture Load - RF-Assisted Alkaline Pretreated Samples

7.4.2.1 Durability Rating

The disruption and deconstruction resulting from the interaction between NaOH solution and the biomass matrix in the presence of RF heating assisted in improving the binding characteristics. The disruption and deconstruction of the lignified matrix is also associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF radiation and the ions and molecules from the NaOH solution and the biomass (Iroba et al. 2013). During the alkaline pretreatment process, the ester bonds between lignin and the complex carbohydrates (cellulose and hemicellulose) are disrupted, some portion of lignin is broken down, degraded, and perhaps solubilised (Iroba et al. 2013). It also enhanced compressing the pretreated ground barley straw sample upon application of pressure and heat at a predefined residence time. Table 7.3 shows the durability, tensile strength, and the fracture load values of the produced pellets. RF-assisted alkaline pretreatment with 1% w/v NaOH concentration produced pellets with higher durability, tensile strength, and fracture load as compared to the non-treated ground barley straw. This was a result of the swelling pores created

on the biomass matrix during the RF alkaline pretreatment process, and subsequently made the binder (lignin) easily accessible during the densification process. Statistical analysis shows that the use of NaOH solution significantly affected ($P<0.05$) the quality of the produced pellets. Nlewem and Thrash (2010) reported that the swelling initiated by NaOH created pores on the biomass matrix which helps in enhancing the accessibility of the soluble lignin. The observed data shows that temperature and the ratio of biomass to NaOH solution influences the durability of the produced pellet. Almost in all cases, higher temperature and lower ratio of biomass:NaOH solution resulted in higher durability of the produced pellets. Higher temperature helps in the local melting of some constituents (Tabil 1996), which subsequently enhanced the production of pellets with good physical characteristics. Decreasing the ratio of biomass:NaOH solution (e.g. 1:8) correspondingly enhanced more reactivity and activity within the biomass matrix leading to the subsequent release of the natural binder (soluble lignin). During compression and compaction of biomass, the smaller (fine) particles rearrange and fill in the void space of larger (coarse) particles therefore producing denser and more durable compacts (Tabil 1996). Analysis of variance shows that the interaction between temperature and ratio of biomass:NaOH solution have significant effects ($P<0.05$) on the durability of the produced pellets. Based on the available collected data from this investigation, RF alkaline pretreatment with ratio 1:8 at the three investigated temperatures represented the optimum condition for producing pellets with better durable features. RF assisted alkaline pretreatment increased the durability of produced pellets by up to 57% over that of pellets from non-pretreated biomass.

Table 7.3 Durability, tensile strength, and fracture load of pellets made from RF-alkaline pretreated ground barley straw

Temperature (^{o}C)	NaOH conc. (%)	Biomass:NaOH solution ratio	Durability (%)	Tensile strength (MPa)	Fracture load (N)
24^{NT}	-	-	63.37 (3.40)^{a}	0.27 (0.04)^{A}	7.11 (1.01)^{f}
	1	1:6	65.63 (2.68)^{a}	0.82 (0.12)^{B}	21.57 (3.08)^{g}
	1	1:7	72.64 (5.61)^{c}	0.86 (0.12)^{B}	22.76 (3.26)^{g}
70	1	1:4	65.80 (3.55)^{a}	0.67 (0.14)^{A}	17.54 (3.77)^{f}
	1	1:5	65.92 (4.39)^{a}	0.87 (0.13)^{AC}	22.92 (3.31)^{g}
	1	1:6	66.22 (2.27)^{a}	0.64 (0.10)^{A}	16.89 (2.68)^{f}
	1	1:7	70.34 (3.60)^{b}	0.99 (0.12)^{BC}	26.09 (3.28)^{g}
	1	1:8	91.37 (4.59)^{c}	1.30 (0.15)^{D}	34.19 (4.01)^{h}
80	1	1:4	66.44 (4.17)^{ab}	0.68 (0.12)^{AB}	17.92 (3.19)^{f}
	1	1:5	68.67 (5.87)^{ad}	0.95 (0.10)^{AC}	25.01 (2.66)^{gik}
	1	$1:5^{W}$	64.10 (2.96)^{bf}	0.45 (0.0)^{BE}	11.76 (1.84)^{h}
	1	1:6	68.59 (2.40)^{ad}	0.91 (0.08)^{C}	24.04 (2.17)^{gk}
	1	1:7	71.09 (2.20)^{d}	1.06 (0.11)^{C}	27.98 (2.87)^{i}
	1	$1:7^{W}$	71.99 (2.92)^{d}	0.80 (0.11)^{C}	20.92 (2.85)^{j}
	0	$1:7^{DW}$	60.36 (1.60)^{e}	0.83 (0.10)^{C}	21.82 (2.55)^{jk}
	0	$1:7^{TW}$	62.79 (1.87)^{f}	0.39 (0.07)^{E}	10.20 (1.91)^{h}
	1	1:8	85.99 (5.56)^{g}	1.39 (0.13)^{F}	36.68 (3.31)^{l}
90	1	1:4	57.19 (4.23)^{a}	0.62 (0.13)^{A}	16.23 (3.34)^{fh}
	1	1:5	71.31 (3.87)^{b}	0.91 (0.10)^{BC}	23.94 (2.55)^{g}
	1	$1:5^{W}$	64.69 (3.18)^{c}	0.52 (0.05)^{A}	13.58 (1.42)^{f}
	1	1:6	71.67 (3.19)^{b}	0.75 (0.11)^{BD}	19.65 (2.90)^{h}
	1	1:7	82.97 (3.18)^{d}	1.03 (0.13)^{C}	27.09 (3.43)^{i}
	1	$1:7^{W}$	78.61 (1.98)^{e}	0.63 (0.09)^{AD}	16.51 (2.44)^{fh}
	1	1:8	99.17 (0.40)^{f}	1.67 (0.19)^{E}	43.82 (4.92)^{j}

Value in parenthesis is standard error; n = 10; Conc. = concentration; W = washed; DW = distilled water; TW = tap water; NT = non-treated biomass; Means with the same superscript letter within the same set (same temperature) in the same column are not significantly different with respect to ratio of biomass:NaOH solution using Tukey's Mean Comparison Test. The mean difference is significant at the 5% significance level.

7.4.2.2 Tensile Strength and Fracture Load

The already released or broken down lignin components assisted in the particle binding mechanisms and behavior resulting in higher tensile strength and fracture load. The softening of the RF alkaline pretreated sample enhanced the creation of the mechanical interlocking of the

biomass particles and supported the strength of the bonds between the adhering partners upon application of pressure and temperature. Kashaninejad and Tabil (2010) reported that pellets made from barley straw pretreated with microwave technique with 1% w/v NaOH have higher tensile strength and fracture load compared to the non-treated samples. The authors reported that the pellets have 2.36 MPa and 53.63 N as tensile strength and fracture load, respectively. These researchers used biomass:NaOH solution ratio of 1:9 as against the lowest ratio of 1:8 used in this present investigation, this may account for the difference in the tensile strength and fracture load values. The samples pretreated with water (distilled or tap) have lower tensile strength and fracture load. This is due to the hydrophobic nature of the lignin matrix, which implies that just water and heat are not enough to breakdown the lignocellulosic matrix. The ratio of biomass:NaOH solution affects the tensile strength and fracture load of the pellets. The lower the ratio, the better the tensile strength and fracture load. RF alkaline pretreatment increased the tensile strength and fracture load of produced pellets by up to 518% and 516%, respectively, over that of pellets from non-pretreated biomass. Temperature also plays a vital role on the tensile strength and fracture load, which depends on the interaction with the ratio of biomass:NaOH solution. Analysis of variance performed on the data shows that temperature, ratio of biomass to NaOH solution, and the interaction between the two variables have significant effects ($P<0.05$) on the tensile strength and fracture load of the produced pellets. Ratio 1:8 at the three investigated temperatures represents the optimum yield of pellet with better compressive strength and fracture load.

Iroba et al. (2013) reported that the use of 1% w/v NaOH solution increased the ash content of the RF pretreated biomass by about 60 to 140% depending on the ratio of biomass to NaOH solution. The ash content increased with the decreasing ratio of biomass to NaOH solution. This is primarily due to the high concentration of mineral content of sodium in the NaOH solution at lower ratio. This problem of increased ash content can be addressed by washing the pretreated samples. Washing the pretreated samples reduced the ash content by about 25-55% depending on the ratio of biomass to the NaOH solution. However, washing of the RF pretreated samples reduced the durability, tensile strength, and fracture load of the produced pellets, which may be a result of loss of the soluble lignin (biomass binding agent) during the washing process. It reduced

the durability and strength of the pellets by about 5.26-9.28% and 25-53%, respectively, depending on the treatment combinations.

7.4.3 Durability, Tensile Strength, and Fracture Load-Steam Explosion Pretreated Samples

Table 7.4 shows the durability, tensile strength, and the fracture load values of the pellets produced from the steam exploded and non-treated biomass. The observed data shows that temperature and retention time are important process conditions for the physical characteristics of the pellets. The higher the temperature and retention time, the higher the durability, tensile strength, and fracture load of the pellets. High temperature and long retention time helped in the local melting of some constituents (such as lignin), which subsequently enhanced the production of pellets with good physical characteristics. The analysis of variance shows that temperature significantly affected ($P<0.05$) the durability of the pellets. Temperature, retention time, and the interaction between temperature and retention time have significant effect ($P<0.05$) on the tensile strength and fracture load of the produced pellets. At lower temperature ($140^{o}C$-$160^{o}C$), increase in the moisture content tended to decrease the quality of the pellets because it takes longer time and higher energy to evaporate the moisture before the local melting of some constituents. However, at higher temperature ($180^{o}C$) increase in the moisture content contribute positively to the durability, tensile strength, and fracture load of the produced pellets. This may be due to higher temperature enhances higher removal rate of moisture from the pretreated biomass, as such the optimum moisture (8-10% wet basis) of the pretreated biomass was achieved, which is needed for a good interlocking/binding of the charged particles to take place, as well as avoiding spring back effect. While at lower temperature, the moisture content of the pretreated biomass was higher than the optimal, therefore, resulting in a lower physical property of the produced pellets.

Based on the available data collected from this investigation, steam explosion pretreatment increases the durability, tensile strength, and fracture load of the produced pellets by up to 22%, 488%, and 481%, respectively, over that of pellets from non-pretreated biomass.

Table 7.4 Durability, tensile strength, and fracture load of pellets made from steam explosion pretreated ground barley straw

Temperature (^oC)	Moisture content (%)*	Retention time (min)	Durability (%)	Tensile strength (MPa)	Fracture load (N)
24^{NT}	8	-	60.01 (3.07)	0.34(0.06)	9.06 (1.45)
140	8	5	60.64 (1.42)^{a}	0.34 (0.05)^{A}	9.00 (1.24)^{f}
	30	5	58.99 (3.31)^{a}	0.29 (0.04)^{A}	7.57 (0.96)^{f}
	50	5	55.46 (4.43)^{a}	0.28 (0.03)^{A}	7.40 (0.82)^{f}
	8	10	61.99 (3.97)^{a}	0.45 (0.07)^{A}	11.87 (1.90)^{f}
	30	10	63.01 (3.29)^{a}	0.31 (0.02)^{A}	8.50 (0.39)^{f}
	50	10	58.17 (4.06)^{a}	0.42 (0.07)^{A}	11.07 (1.72)^{f}
160	8	5	62.08 (4.28)^{ad}	0.39 (0.03)^{A}	9.70 (0.87)^{f}
	30	5	59.97 (2.20)^{a}	0.32 (0.07)^{A}	9.03 (1.85)^{f}
	50	5	59.96 (4.65)^{a}	0.33 (0.08)^{A}	9.39 (2.00)^{f}
	8	10	72.64 (4.04)^{b}	0.67 (0.14)^{B}	17.62 (3.57)^{g}
	30	10	66.13 (3.30)^{cd}	0.46 (0.08)^{C}	11.99 (2.08)^{f}
180	8	5	66.23 (4.82)^{a}	0.61 (0.11)^{AC}	16.16 (2.89)^{f}
	30	5	68.13 (5.61)^{a}	0.95 (0.11)^{BD}	24.90 (2.95)^{gh}
	50	5	70.77 (3.41)^{ac}	0.78 (0.10)^{CD}	20.53 (2.73)^{fg}
	8	10	73.03 (4.27)^{bc}	1.00 (0.13)^{D}	26.39 (3.42)^{h}
	30	10	69.28 (5.12)^{ac}	1.57 (0.17)^{E}	41.29 (4.49)^{i}
	50	10	72.39 (3.08)^{c}	2.00 (0.25)^{F}	52.61 (6.59)^{k}

Value in parenthesis is standard error; n = 10; * = % mass fraction of total mass; NT = non-treated biomass; Means with the same superscript letter within the same set (same temperature) in the same column are not significantly different with respect to moisture content and retention time using Tukey's Mean Comparison Test. The mean difference is significant at the 5% significance level.

7.4.4 Pellet Density and Dimensional Stability–RF Assisted Alkaline Pretreated Samples

Table 7.5 shows the density, % density change, and dimensional stability values of the pellets produced from the RF assisted alkaline pretreated and non-treated samples. Samples pretreated with 1% w/v NaOH concentration have higher density than the non-pretreated sample. This is due to the released of the binding agent (lignin) which increased the adhesion among the particles, favored the generation of the intermolecular bonds within the contact area of the biomass particles, and subsequently enhances the mechanical interlocking of the particles. A similar result was reported by Kashaninejad and Tabil (2010). The authors reported that the

pellets have 1371 kg/m^3 and 995 kg/m^3 as the initial density of the microwave alkaline pretreated and non-treated barley straw grind, respectively. These researchers used 1% NaOH with ratio 1:9 of biomass to NaOH solution. When biomass is subjected to heat, lignin tends to become soft, melts and exhibits thermosetting binder resin properties to produce pellets with higher density and dimensional stability (Tabil 1996). The pretreatment temperature and ratio of biomass:NaOH solution played a predominant role in producing pellets with high density. The three investigated temperatures (70-90^{o}C) and low biomass:NaOH solution ratio (1:8) resulted to higher pellet density. Analysis of variance shows that temperature, ratio of biomass:NaOH solution and the interaction between the two variables have significant effects ($P<0.05$) on the density and dimensional stability of the produced pellets. Washing of the pretreated samples also seems to decreased the pellet density. This might be also due to the reason that the available released soluble lignin was washed away during the washing process. The positive and negative % density change in Table 7.5 corresponds to diametric or longitudinal expansion and contraction, respectively, of the pellets after two weeks of storage. Pellets with diametric or longitudinal expansion resulted in density decrease, while pellets with diametric or longitudinal contraction led to density increase. The pellets density was obtained from mass per unit volume, and volume was calculated from the diameter and height of the pellets ($\pi r^2 h$). Therefore, any change in diameter and the height will have a corresponding effect on the density, which also translated into the dimensional stability of the produced pellets. The lesser the % density change, the better the dimensional stability of the produced pellets. The % density change and dimensional stability showed that RF alkaline pretreatment was effective at all the temperatures and ratios studied. The density increase was about 9% to 35% over that of pellets from non-pretreated biomass, with percentage density change of -2.93% to 2.26%, and 97.74% to 102.93% dimensional stability. This implies that all the pellets produced from the RF alkaline pretreated samples will present easy, economical, and efficient transportation, handling, storage characteristics (less volume requirements), and utilization as a fuel. They can also withstand the shear, impact, rotation, and tumbling during the transportation stage, without causing wide spread of air pollution in the form of very fine particulate matters as described by Tabil 1996; Mani et al. 2006b; Adapa et al. 2009a; Grover and Mishra 1996). This also implies that RF alkaline pretreatment can enhance the biomass volumetric calorific/heating value per unit volume, which can be easily adopted into

the "direct-combustion or co-firing with coal, gasification, pyrolysis, and biomass-based" conversion reactors (Granada et al. 2002; Kaliyan and Morey 2009b). RF assisted alkaline pretreatment increases the pellet density by up to 35% over that of pellets from non-pretreated biomass. This will further increase the thermal and conversion efficiency by a similar percentage factor. In this current investigation, ratio 1:8 at the three levels of investigated temperatures represented the optimum yield of the pellets density. Treatment combinations of ratio 1:6 and 1:7 at room temperature resulted in producing pellets with good physical quality characteristics. This shows that biomass can be pretreated even at room temperature if the required optimum ratio of biomass:NaOH solution is used with the required pretreatment retention time. From the statistical analysis performed on the collected data, the ratio of biomass:NaOH solution presented to be more significant than temperature on the density, durability, and tensile strength.

Table 7.5 Pellet density, density change, and dimensional stability during 2 weeks of storage of RF alkaline pretreated and non-treated ground barley straw

Temperature (^oC)	NaOH conc. (%)	Biomass: NaOH solution ratio	Density1 (kg/m^3)	Density2 (kg/m^3)	Density change (%)	Dimensional stability (%)
24^{NT}	-	-	1004.49 (1.61)^{a}	995.19 (2.21)^{A}	0.93	99.07
	1	1:6	1230.29 (0.94)^{b}	1220.82 (1.69)^{B}	0.77	99.23
	1	1:7	1252.25 (1.05)^{b}	1244.33 (1.39)^{B}	0.63	99.37
70	1	1:4	1129.82 (2.18)^{a}	1113.24 (2.06)^{A}	1.47	98.53
	1	1:5	1165.09 (1.70)^{a}	1154.87 (1.75)^{A}	0.88	99.12
	1	1:6	1175.70 (1.49)^{a}	1167.52 (1.94)^{B}	0.70	99.30
	1	1:7	1180.15 (12.05)^{a}	1214.68 (7.62)^{C}	-2.93	102.93
	1	1:8	1324.71 (2.14)^{b}	1309.10 (2.31)^{D}	1.18	98.82
80	1	1:4	1154.88 (1.37)^{a}	1142.20 (2.26)^{A}	1.10	98.90
	1	1:5	1222.76 (3.65)^{b}	1212.84 (2.77)^{BC}	0.81	99.19
	1	$1:5^{W}$	1136.82 (1.54)^{a}	1127.16 (2.18)^{A}	0.85	99.15
	1	1:6	1239.29 (1.39)^{b}	1225.19 (1.39)^{B}	1.14	98.86
	1	1:7	1215.34 (3.30)^{bc}	1213.83 (2.25)^{BC}	0.12	99.88
	1	$1:7^{W}$	1171.33 (5.31)^{ac}	1174.08 (3.78)^{AC}	-0.23	100.23
	0	$1:7^{DW}$	1007.50 (2.13)^{d}	1000.45 (1.78)^{D}	0.70	99.30
	0	$1:7^{TW}$	989.95 (3.33)^{d}	974.79 (2.96)^{D}	1.53	98.47
	1	1:8	1342.82 (1.24)^{e}	1326.98 (1.69)^{E}	1.18	98.82
90	1	1:4	1108.69 (3.46)^{a}	1083.59 (2.11)^{A}	2.26	97.74
	1	1:5	1201.40 (3.79)^{b}	1213.48 (1.70)^{B}	-1.01	101.01
	1	$1:5^{W}$	1205.89 (2.58)^{b}	1178.69 (2.84)^{B}	2.26	97.74
	1	1:6	1180.15 (3.39)^{b}	1167.54 (3.08)^{B}	1.07	98.93
	1	1:7	1270.22 (2.53)^{c}	1263.88 (2.01)^{C}	0.50	99.50
	1	$1:7^{W}$	1205.81 (1.45)^{b}	1200.35 (1.59)^{B}	0.45	99.55
	1	1:8	1358.39 (1.34)^{d}	1342.91 (2.02)^{D}	1.14	98.86

Value in parenthesis = standard error; n = 18, NT = non-treated; Conc. = concentration; Density1 = density of pellets immediately after the pelleting, & Density2 = density of pellets after two weeks of storage at room condition; Means with the same superscript letter within the same set (same temperature) in the same column are not significantly different with respect to ratio of biomass:NaOH solution using Tukey's Mean Comparison Test. The mean difference is significant at the 5% significance level.

7.4.5 Pellet Density and Dimensional Stability - Steam Explosion Pretreated Samples

Similar changes were observed in the density and dimensional stability of pellets produced from the SE pretreated samples. However, the pellets density produced from the steam exploded samples are not as high as the pellets density produced from the RF-assisted alkaline pretreated samples. The pretreatment temperature and retention time play a pre-dominant role in producing pellets with good physical properties. The higher the temperature and retention time, the higher were the pellet density and dimensional stability values. At higher temperature, there is a characteristic softening and exhibition of thermosetting binder resin properties in biomass that enhances the production of pellets with good physical qualities (Tabil 1996). Statistical analysis shows that temperature, retention time, and the interaction of both variables significantly affected ($P<0.05$) the density and dimensional stability of the produced pellets. Increasing the moisture content at lower temperature (140-160^{o}C) decreases the density and dimensional stability of the produced pellets. High moisture at lower temperature tends to create a buffer effects, damp the biomass and prevent lignin from getting to it melting point. However, increasing the moisture content at higher temperature (180^{o}C) led to higher density and dimensional stability. This implies that optimum window of moisture content of biomass is required for the manufacture of dimensionally stable and high density pellet. At a required temperature, water acts as a binding agent by strengthening the bonding in the material. Water helps to develop van der Waals forces by increasing the area of contact between particles (Grover and Mishra 1996). Analysis of variance shows that moisture content and the interaction of moisture content, temperature, and retention time significantly affected ($P<0.05$) the density and dimensional stability of the produced pellets. The density increase from the non-pretreated biomass sample to the steam explosion pretreated was about 25%.

Table 7.6 Pellet density, density change, and dimensional stability during 2 weeks of storage of steam exploded pretreated and non-treated ground barley straw

Temperature (^oC)	Moisture content (%)*	Retention time (min)	Density1 (kg/m^3)	Density2 (kg/m^3)	Density Change (%)	Dimensional stability (%)
24^{NT}	8	-	942.57 (9.68)	907.46 (9.34)	3.72	96.28
140	8	5	944.32 (3.88)^{a}	928.83 (4.46)^{A}	1.64	98.36
	30	5	906.70 (8.57)^{a}	862.68 (7.60)^{B}	4.86	95.14
	50	5	936.80 (9.66)^{a}	843.99 (6.14)^{B}	9.91	90.09
	8	10	958.93 (3.70)^{a}	929.35 (5.87)^{A}	3.08	96.92
	30	10	906.59 (5.86)^{a}	828.31 (8.76)^{B}	8.63	91.37
	50	10	932.10 (5.57)^{a}	851.29 (11.01)^{B}	8.67	91.33
160	8	5	898.57 (10.52)^{a}	856.73 (13.30)^{A}	4.66	95.34
	30	5	954.63 (3.44)^{a}	937.69 (3.56)^{B}	1.77	98.23
	50	5	958.02 (3.74)^{a}	887.15 (5.15)^{AB}	7.40	92.60
	8	10	1035.05 (6.08)^{b}	1020.26 (6.84)^{C}	1.43	98.57
	30	10	938.57 (7.20)^{a}	862.07 (8.15)^{A}	8.15	91.85
180	8	5	1040.80 (9.56)^{a}	1005.86 (7.20)^{A}	3.36	96.64
	30	5	1086.45 (4.74)$_{a}$	1038.58 (5.02)^{A}	4.41	95.59
	50	5	1060.89 (3.55)^{a}	1011.87 (7.91)^{A}	4.62	95.38
	8	10	1177.38 (3.65)^{b}	1147.04 (4.46)^{B}	2.58	97.42
	30	10	1174.35 (3.49)^{b}	1150.87 (3.75)^{B}	2.00	98.00
	50	10	1152.08 (5.47)^{b}	1146.55 (5.88)^{B}	0.48	99.52

Values in parentheses is standard error; n = 10; * = % mass fraction of total mass; NT = non-treated biomass; Means with the same superscript letter within the same set (same temperature) in the same column are not significantly different with respect to moisture content and retention time using Tukey's Mean Comparison Test. The mean difference is significant at the 5% significance level.

7.4.6 Color Analysis

7.4.6.1 Radio Frequency-Alkaline Pretreated Samples

Table 7.7 shows the mean values of the color parameters of L*, a*, and b* of the RF-alkaline pretreated and non-treated ground barley straw. Color characteristics of the pretreated samples are through reflection of the degree of the pretreatment conditions. The alkaline solution and ratio of biomass:NaOH solution are the key variables responsible for the color changes. The degree of lightness (L*) and yellowness (b*) decreased as the ratio of biomass:NaOH solution decreases from 1:4 to 1:8, indicating that the samples become more brownish. Increasing

temperature increased the darkness and blueness of the samples; however, this was not the case for all the treatment combinations.

Table 7.7 Color characteristics of radio frequency alkaline and non-treated ground barley straw

Temperature (^{o}C)	NaOH conc. (%)	Biomass: NaOH solution ratio	L*	a*	b*	ΔE*
24^{NT}	-	-	63.22 (0.25)	7.32 (0.01)	21.90 (0.12)	-
	1	1:6	54.17 (0.01)	7.64 (0.01)	34.44 (0.01)	15.47 (0.25)
	1	1:7	53.62 (0.02)	7.90 (0.00)	34.31 (0.02)	15.70 (0.25)
70	1	1:4	54.45 (0.01)	7.71 (0.00)	35.14 (0.00)	15.89 (0.24)
	1	1:5	51.98 (0.01)	7.75 (0.00)	33.79 (0.01)	16.37 (0.26)
	1	1:6	51.88 (0.00)	8.07 (0.00)	33.61 (0.01)	16.32 (0.26)
	1	1:7	50.38 (0.01)	8.45 (0.00)	33.60 (0.01)	17.41 (0.27)
	1	1:8	50.99 (0.01)	8.52 (0.01)	33.72 (0.01)	17.05 (0.26)
80	1	1:4	54.60 (0.01)	7.81 (0.01)	33.96 (0.01)	14.83 (0.24)
	1	1:5	52.97 (0.01)	7.81 (0.00)	34.68 (0.01)	16.39 (0.25)
	1	$1:5^{W}$	61.55 (0.00)	6.61 (0.00)	30.10 (0.01)	8.40 (0.18)
	1	1:6	50.79 (0.01)	8.60 (0.00)	33.94 (0.01)	17.35 (0.27)
	1	1:7	51.11 (0.00)	8.46 (0.01)	33.61 (0.01)	16.88 (0.26)
	1	$1:7^{W}$	60.71 (0.01)	6.19 (0.00)	30.46 (0.03)	9.00 (0.21)
	0	$1:7^{DW}$	58.45 (0.33)	7.43 (0.00)	26.40 (0.02)	3.05 (0.26)
	0	$1:7^{TW}$	60.24 (0.01)	7.65 (0.01)	22.45 (0.01)	6.57 (0.44)
	1	1:8	50.12 (0.01)	8.73 (0.01)	33.68 (0.03)	17.67 (0.27)
90	1	1:4	53.04 (0.01)	8.00 (0.10)	33.89 (0.41)	15.75 (0.06)
	1	1:5	51.32 (0.01)	8.21 (0.00)	34.14 (0.02)	17.10 (0.26)
	1	$1:5^{W}$	60.32 (0.01)	6.37 (0.01)	31.14 (0.02)	9.73 (0.20)
	1	1:6	51.51 (0.01)	8.26 (0.00)	33.52 (0.00)	16.52 (0.26)
	1	1:7	50.39 (0.01)	8.83 (0.00)	32.66 (0.00)	16.81 (0.27)
	1	$1:7^{W}$	60.79 (0.00)	6.36 (0.01)	29.45 (0.03)	7.99 (0.22)
	1	1:8	49.60 (0.02)	8.61 (0.01)	32.95 (0.01)	17.58 (0.26)

Value in parentheses = standard error; number of replicates, n = 3; conc. = concentration; The scale L* represents from 0 for black to +100 for white; The a* and b* have no specific numerical limits; Positive a* is red; Negative a* is green; Positive b* is yellow; Negative b* is blue; and ΔE* = total color difference.

Washing the RF alkaline pretreated samples increases the lightness and yellowness of the samples. The redness (a*) of the RF alkaline pretreated samples increased with decreasing ratio of biomass:NaOH solution and increasing temperature. Washing decreases the redness of the samples and makes it to become greener. The lightness (L*) of the non-treated samples are higher but lower for the scale parameter a* and b* compared to the RF alkaline pretreated samples. The total color difference (ΔE*) between the RF alkaline pretreated and non-treated samples increases with decreasing ratio of biomass:NaOH solution; however, this was also not the case for all the treatment combinations. The effect of temperature on color difference is not as much as that of ratio. Washing reduces the total color difference.

7.4.6.2 Steam Explosion Pretreated Samples

Table 6.8 shows the mean values of the color parameters of L*, a*, and b* of the steam exploded and non-treated samples. The collected data indicates that color characteristics of the pretreated samples are associated with the severity of the pretreatment conditions. The lightness (L*) of steam exploded ground barley straw decreased with increasing temperature and retention time. This implies that, the higher the severity of the pretreatment conditions, the darker the pretreated samples. In most cases, the yellowness of the steam exploded samples decreased with increasing temperature and retention time, except for samples pretreated at 160^{o}C, moisture content of 8-30% (mass fraction of water) and at 5-10 min retention time. Increasing blueness also signifies that the samples get darker as the severity of pretreatment increased with respect to increasing temperature and retention time. The lightness (L*) and yellowness (b*) of the non-treated ground barley straw are higher as compared to the steam pretreated samples. The calculated differential color composite ΔE* is a good indicator of color deviation between pretreated and non-treated samples. The total color difference between the steam exploded and non-treated samples increases with increasing temperature and retention time. The highest total color difference was observed at 180^{o}C. Zhang and Cai (2006) reported that the color changes observed in wood during steam treatment were as a result of series of chemical reactions. These are primarily due to the "reactions between the chemical constituents of wood cell wall and extractives under high temperature and humidity conditions". Polysaccharides (cellulose and hemicellulose) can cause

extreme changes at a critical temperature of 120^{o}C. Cellulose degrades to yellow when the temperature is above 140^{o}C (Zhang and Cai 2006).

Table 7.8 Color characteristics of steam exploded and non-treated ground barley straw

Temperature (^{o}C)	Moisture content (%)*	Retention time (min)	L*	a*	b*	ΔE*
NT	8	-	60.83 (0.01)	7.67 (0.01)	20.36 (0.02)	-
140	8	5	55.82 (0.01)	8.31 (0.01)	16.97 (0.01)	6.08 (0.01)
160	8	5	54.68 (0.00)	9.13 (0.01)	18.24 (0.01)	6.67 (0.02)
180	8	5	29.90 (0.02)	7.23 (0.03)	8.24 (0.02)	33.22 (0.02)
140	30	5	57.10 (0.01)	8.74 (0.00)	20.29 (0.02)	3.88 (0.01)
160	30	5	47.60 (0.00)	9.03 (0.01)	20.11 (0.01)	13.30 (0.01)
180	30	5	32.93 (0.02)	9.08 (0.00)	13.35 (0.02)	28.80 (0.00)
140	50	5	50.37 (0.00)	9.01 (0.00)	19.16 (0.01)	10.61 (0.01)
160	50	5	41.78 (0.00)	8.28 (0.01)	15.65 (0.01)	19.63 (0.01)
180	50	5	31.59 (0.01)	6.75 (0.01)	7.40 (0.02)	32.00 (0.02)
140	8	10	53.15 (0.01)	9.20 (0.00)	19.34 (0.00)	7.89 (0.01)
160	8	10	45.90 (0.01)	10.18 (0.01)	17.24 (0.01)	15.46 (0.01)
180	8	10	30.98 (0.00)	9.18 (0.01)	12.45 (0.02)	30.91 (0.01)
140	30	10	51.07 (0.00)	9.23 (0.01)	17.68 (0.01)	10.24 (0.01)
160	30	10	50.99 (0.01)	9.58 (0.00)	19.16 (0.01)	10.09 (0.01)
180	30	10	30.44 (0.01)	8.01 (0.01)	8.53 (0.01)	32.62 (0.01)
140	50	10	47.65 (0.01)	9.25 (0.00)	17.94 (0.00)	13.49 (0.01)
180	50	10	30.41 (0.01)	6.58 (0.01)	7.14 (0.00)	33.18 (0.01)

Value in parentheses = standard error; number of replicates, n = 3; * = % mass fraction of total mass; The scale L* represents from 0 for black to +100 for white; The a* and b* have no specific numerical limits; Positive a* is red; Negative a* is green; Positive b* is yellow; Negative b* is blue; and ΔE* = total color difference.

From the collected data in this current study, hemicellulose is more sensitive to temperature and easier to degrade than cellulose. During steam treatment furfural and some polysaccharides with low molecular weights are generated from the degradation of hemicellulose. Changes in lignin and extractives can also contribute to the color changes. Carboxylates and phenol may be produced within the lignin or extractive molecules at high temperatures and humidity (Zhang and Cai 2006). All these put together might lead to color changes in the appearance of the ground barley straw during steam explosion pretreatment.

7.5 Conclusion

The present study shows several strategies to improve the durability of barley straw pellets. Radio frequency-alkaline pretreatment using 1% NaOH solution with the ratio of biomass:NaOH solution enhanced the deconstruction of the lignocellulosic matrix in barley straw. The lower was the biomass:NaOH solution ratio, the better was the quality of the produced pellets. Washing of the RF-alkaline pretreated samples resulted in pellets with low quality. A biomass:NaOH solution ratio of 1:8 at the three levels of temperature (70, 80, and 90^{o}C) studied are the RF optimum pretreatment conditions for the production of pellet with better pellet strength, pellet fracture load, density, durability rating, and dimensional stability. The operating temperature and retention time represent two important variables in the steam explosion pretreatment that contributed significantly to the production of pellets with good physical properties. Higher temperature (180^{o}C), 10 min retention time at the three levels of moisture content are the optimum pretreatment condition that resulted in pellets with better physical quality compared to those from non-treated barley straw. Therefore, this study confirmed that lignocellulosic biomass has a natural binder (lignin) which acts as an adhesive/glue that binds the biomass particles during densification process. Hence, there is a need to fully or partially break or disrupt the lignocellulosic matrix in the biomass via pretreatment before densification. In comparison with steam explosion, RF-assisted alkaline pretreatment of ground barley straw produced pellets with better physical characteristics. However, this combined approach (radio frequency in combination with alkaline pretreatment) involved longer pretreatment time compared to steam explosion pretreatment.

Chapter 8

8. General Discussion

This chapter is aimed at the overall general discussion of the results obtained from this thesis and how the research objectives listed in Chapter 1 (Section 1.10) have been achieved over the course of the Ph.D. project.

8.1 Overall Ph.D. Project Discussion

This Ph.D. thesis explored the effects of pretreatment techniques on both the chemical compositions and densification quality parameters of lignocellulosic biomass barley straw grind. Radio frequency dielectric heating using alkaline as a catalyst and steam explosion at low severity factor were the pretreatment approaches employed in this study. The acid soluble lignin, acid insoluble lignin, cellulose (glucose), hemicellulose (xylose, galactose, mannose, and arabinnose) were the chemical properties studied, while density, durability rating, tensile strength, and dimensional stability were the pellet quality characteristics investigated.

The structural representation of lignocellulosic biomass is characterized by the association of inaccessible polysaccharides (cellulose and hemicellulose) and lignin, which acts as a barrier to any external influence. The highly organized crystalline structure of cellulose always acts as obstacle to hydrolysis. Cellulose and hemicellulose are broadly protected from enzymatic attack. As such biodegradation of non-treated lignocellulosic biomass will result in low yield of simple sugars from enzymatic hydrolysis. To achieve higher conversion rate, the enzymatic process will require relatively high enzyme loadings (high cost) to generate monomeric simple sugars (glucose, xylose, galactose, mannose, and arabinose) that are readily fermentable by microorganisms (yeasts). This makes the process economically unfeasible.

Therefore, application of pretreatment and preprocessing is required to disintegrate and disrupt lignocellulosic structure of biomass, and cause relative changes in the composition of cellulose, hemicelluloses, and lignin content in the biomass material. Pretreatment is generally aimed at breaking down the lignocellulosic matrix, loosening the highly crystalline structure of cellulose, increases the porosity of the biomass, and enhance the digestibility and accessibility of cellulose and hemicellulose.

Modification of the structural arrangement of cellulose-hemicellulose-lignin matrix can improve the natural binding characteristics of the lignocellulosic barley straw. This biomass natural binder (lignin) can be harnessed through pretreatment process. Therefore, pretreatment of biomass makes it suitable for densification. To mitigate the difficulty resulting from the loose, bulky, and disperse nature of lignocellulosic biomass, it is therefore necessary that they are preprocessed, pretreated, and densified for easy, economical and efficient handling, storage, and transportation. It increases mass per unit volume and improves the convenience and accessibility of lignocellulosic biomass feedstock as a result of the uniform shape and size.

The particle and bulk densities increased as the hammer mill screen size of the biomass decreased. The effect of moisture transfer and compaction during the grinding process may be responsible for the variation of density with biomass hammer mill screen size.

The dielectric properties of lignocellulosic biomass barley straw and sodium hydroxide relevant to radio frequency pretreatment processes were measured (Appendix A). The effects of the process temperature, biomass moisture content, sodium hydroxide concentration, biomass screen size, soaking time, and ratio of biomass to alkaline solution on dielectric constant, dielectric loss factor, and power penetration depth were investigated. The dielectric constant and dielectric loss factor increased with increasing ratio of biomass to NaOH solution until it reached maximum and decreased at the critical ratio R_c. The critical ratio depends on the operating temperature. The power penetration depth has inverse relationship with all the material and process variables tested. It decreased as any of the material or process variables increases. Regression equations were developed to relate the dielectric properties to temperature, particle size, NaOH

concentration, soaking time, and ratio of biomass to alkaline/water solution. Such equations can be applied by the biofuel industry to improve process efficiency during design by eliminating the need for actual experimental procedure. The obtained data was used to established relationships among the tested variables and subsequently applied in the design, formulation, and optimization of the functional RF pretreatment process used in chapter 3.

The addition of NaOH to lignocellulosic biomass during the RF pretreatment causes swelling of particles within the mixture. This swelling is a result of the saponification of intermolecular ester bonds linkages within the biomass. It enhances the penetration of enzymatic and microbiological activities into the cell-wall fine structure of lignocellulosic biomass. Subsequently, it assists in the separation of the structural linkages between lignin and the polysaccharides, increases the porosity of lignocellulosic biomass, and results to an increase in internal surface area. Lignocellulosic biomass absorbs more NaOH than water, because of the hydrophobic nature of lignin, which acts as an external crosslink binder on the biomass matrix and shields the hydrophilic structural carbohydrates (cellulose and hemicellulose).

There was reduction in the acid insoluble lignin in the RF-alkaline pretreated samples as compared to the non-treated samples. Lignin removal is an important part of the pretreatment process, because lignin can effectively inhibit/prevent the cellulase enzymes from hydrolyzing the cellulose. This solid loss of lignin was believed to be associated with the structural separation, disruption and disaggregation initiated by the interaction between the biomass and NaOH solution in the presence of the RF heating. The disruption and deconstruction of the lignified matrix is also associated with the dipole interaction, flip flop rotation, and friction generated between the electromagnetic charges from the RF and the ions and molecules from the NaOH solution and the biomass.

The use of NaOH solution and decreasing the ratio of biomass to the NaOH solution increased the total acid soluble lignin moieties. Easy access to the total acid soluble lignin moieties is associated with the breakdown of the lignified biomass matrix, due to the already initiated pore swelling during the RF-assisted alkaline pretreatment process. This reflects enhancement of the

accessibility and digestibility of the cellulose and hemicellulose. A biomass to NaOH solution ratio of 1:6 resulted in the maximal effect of pretreatment, with yields of the total acid soluble lignin moieties 31-64% greater than from the non-treated biomass. This will assist in reducing the costs and amount of enzymes required for the next stage of process (enzymatic hydrolysis) by about 64% compared to the non-treated biomass.

The lignin in the raw sample is more difficult to solubilize during the chemical composition analysis, because the lignified matrix is tightly bound to the other constituents. The preserved cellulose and hemicellulose from the raw sample (non-treated) are higher than that from the RF-alkaline pretreated samples because of the initial degradation of the sugars during the pretreatment process. This implies that there is a trade-off between the breakdown of the biomass matrix/creating pores in the lignin and enhancing the accessibility and digestibility of the cellulose and hemicellulose. The more the biomass matrix (lignin bond) is broken down, the more the components of interest (cellulose and hemicellulose) were degraded.

In general, as earlier mentioned, the treatment combinations using the biomass to NaOH solution ratio of 1:6 at all temperatures studied resulted in optimum yields of the cellulose and hemicellulose and total acid soluble lignin moieties. At optimal conditions, pretreatment enhanced hemicellulose yields by 75-97%, compared to enhancements of 67-75% yield for cellulose. Hemicellulose had higher yield because of its random, branched, and amorphous structure with little strength unlike the crystalline, highly ordered cellulose with a high degree of polymerisation that requires more severe pretreatment conditions.

This study shows that the concentration of NaOH and the ratio of biomass to the NaOH solution are the dominant contributing parameters, while temperature plays a lesser role.
The use of NaOH solution increased the ash content of the pretreated biomass. The ash content increased with the decreasing ratio of biomass to NaOH solution due to the high concentration of mineral content of sodium in the NaOH solution at lower ratio. This problem of increased ash content can be addressed by washing the pretreated samples.

It is expected that this RF pretreatment and chemical composition test procedure developed in this thesis will be a stepping stone and initiate a drive towards the application of RF in the biomass pretreatment strategies. This will enhance post-harvest processing of agricultural biomass, improve process efficiency, and subsequently bring economic benefits to the biofuel industry. In addition, the calculated heating rate and energy consumption during the RF-alkaline pretreatment can assist in determining the economic feasibility of pretreatment process. The available RF power level for commercialization is now up to several hundred kW.

The steam explosion pretreatment of lignocellulosic biomass at low severity factor demonstrated that the severity factor increased with increasing temperature and time. Moisture content of biomass acts as a buffer to dampen the severity of the pretreatment condition, and subsequently moderate the formation of inhibitors such as furfural. The acid insoluble lignin (AIL) increased as the temperature increased. This may be attributed to the carbonization of the sample resulting from the direct contact of biomass with the walls of the reactor. At a higher temperature (180°C), exothermic reaction occurred, leading to the degradation of cellulose and hemicellulose. Hemicellulose degrades easily and some volatile organic compounds vaporize as volatile components, while cellulose behaves as a fixed carbon (solid combustible residue). The degraded cellulose appears as residue, thereby accounting for the increase in the AIL content. The percentage AIL increase was between 1.61 to 91.28% relative to the non-treated biomass. This may also be accounted for by lignin's high resistance to thermal degradation during the steam explosion pretreatment. This investigation shows that AIL increased as the moisture content decreased from 50 to 8% (w.b.). This implies that biomass initial moisture content acts as buffer to dampen the severity of the pretreatment process; thereby, partially preventing the degradation of the amorphous portion of the structural carbohydrates that could be deposited as residue and subsequently increasing the quantity of AIL in the treated biomass. At higher temperature and retention time, the total acid soluble lignin moieties content correspondingly increased. At higher temperature and retention times the biomass matrix was deconstructed, which enhanced the assessment of the total acid soluble lignin moieties. The optimum pretreatment condition (180^{o}C, 8% (w.b.), and 10 min retention time) resulted in the maximal effect of pretreatment, with yields of the total acid soluble lignin moieties 33% greater than from the non-treated biomass. Easy

access to the total acid soluble lignin moieties is associated with the breakdown of the lignified biomass matrix. This reflects maximum enhancement of the accessibility and digestibility of the cellulose and hemicellulose by 33% during the enzymatic reaction. This will also help in decreasing the costs and amount of enzymes required for the next stage of enzymatic hydrolysis by about 33% compared to the non-treated biomass.

The ash content in the steam explosion pretreated ground barley straw increased as the temperature and retention time increased. This could also be due to the degradation of the amorphous portion of the hemicellulose and cellulose. Cellulose loss (degradation) was between 58 and 77%, while hemicellulose degradation was between 79 and 89%.

Furfural formation is dependent on the severity of the steam pretreatment environment. The higher the temperature and retention time, the higher the furfural formation. This was attributed to the reactions and degradations of xylose and glucose from hemicellulose and cellulose, respectively. The higher the biomass initial moisture content, the lower the furfural formation, because the system was dampened and subsequently reduced the harsh pretreatment condition that would have led to formation of high degree of inhibitors.

The severity of the steam treatment condition also affects the elemental composition of biomass. Higher severity resulted in increased carbon content of biomass. The initial carbon and oxygen content of the non-treated barley straw was 42.30% and 50.99%, respectively. After the steam pretreatment (at 160-180^{o}C, 8% moisture content (w.b.), and 5 min retention time), the carbon content increased to a maximum of 46.69-46.89%, while the oxygen content decreased to 45.76-46.77%. The higher heating value (HHV) of the steam treated biomass increased with temperature and decreased with increasing moisture content. The HHV increased up to a maximum of about 11% relative to the non-treated biomass. This is a reflection of the increased carbon content with decreased in oxygen content as the temperature increased. The degree of carbonization increased with temperature and slightly decreased with moisture content of the biomass. This also demonstrated that the water present in the biomass can acts as a damper to reduce the severity of the pretreatment conditions. The increase in the carbon content was due to

the carbonization of the sample resulting from the direct contact of biomass with the walls of the reactor. The small inlet opening (about 10 mm) of the reactor and the moist nature of the samples, made loading of the samples into the reactor very difficult. As such, a thin rod was used to push down the samples as they were loaded into the reactor. This resulted in the loaded samples sticking to the walls of the reactor. Since the heat source of the reactor was wrapped round it, the heat transfer was by conduction which comes from outside to inside of the reactor. This resulted in the wall of the reactor having a higher temperature than the inside of the reactor. Color characteristics of the steam explosion pretreated samples are a reflection of the severity of the pretreatment conditions. The lightness (L*) of steam exploded ground barley straw decreased by up to a maximum of about 51% with increasing temperature and retention time. The lightness (L*) and yellowness (b*) of the non-treated ground barley straw were higher relative to the steam pretreated samples. The total color difference between the steam exploded and non-treated samples increased with increasing temperature and retention time. The highest total color difference was observed at 180^{o}C.

Taken cognizance of the severity factor of the steam pretreatment conditions will to a large extent improve the breaking down of the lignified biomass matrix, enhance the solubilization of the acid soluble lignin moieties, limiting the thermal degradation of cellulose and hemicellulose, limits the formation of inhibitors, and increase the higher heating value of the steam pretreated biomass. It is envisioned that this newly developed concept and procedure will contribute significantly to the use of steam explosion within the biofuel industry.

The acid insoluble lignin from the SE process is higher than that from the RF alkaline pretreatment. The increased in the SE pretreated samples was accounted for by the degradation of cellulose and hemicellulose, which appears as residues resulting to the increase in the acid insoluble lignin. The acid soluble lignin moieties, cellulose, and hemicellulose from the RF alkaline pretreated samples are higher than that from the SE samples.

The radio frequency alkaline and steam explosion treatment combinations that resulted to optimum yield of cellulose and hemicellulose were selected and then enzymatically digested

with a combined mixture of cellulase and β-glucosidase enzymes. The glucose in the hydrolyzed samples was subsequently quantified. The average available percentage glucose yield that was released during the enzymatic hydrolysis for bioethanol production ranged from 78 to 96% for radio frequency alkaline pretreated samples and 30 to 50% for the steam explosion pretreated samples, depending on the treatment combination.

It was also observed from this investigation that biomass can be alkaline pretreated even at room temperature if the required ratio of biomass and NaOH solution is applied. However, the percentage digestible glucose yield was not as high as that of RF alkaline pretreated (~ 23% less). Therefore for optimal enzymatic hydrolysis/saccharification and fermentation yield, the lignocellullosic biomass should be RF alkaline pretreated at a regulated temperature. While the non-treated sample has available average percentage glucose yields of just below 12%. The high stability of the non-treated/native lignocellulosic biomass is responsible for the low yield when subjected to enzymatic or bacterial attacks. The biofuel industry can subsequently use this protocol for the quantification of the glucose present in the hydrolyzed samples.

Rapid screening and quantification analysis of the modified compositional and structural arrangement of cellulose-hemicellulose-lignin matrix resulting from pretreatment is necessary to assess the effectiveness of the pretreatment process. The traditional approach (acid hydrolysis followed by analytical and gravimetric determination) of biomass chemical compositional analysis was initially performed as earlier pointed out above. However, this approach is highly time consuming, laborious and costly to perform. Based on the various benefits and advantages (e.g. superior spectral resolution and provides information on the fundamental molecular vibrations) associated with Fourier transform infrared (FTIR), a novel procedure was developed to quantify the lignocellulosic chemical composition using FTIR technique. Subsequently, regression models were developed to rapidly estimate the lignocellulosic biomass content. This procedure could be easily adopted by the biofuel industry and extended to any form of lignocellulosic biomass using FTIR spectroscopy. It is also expected to improve efficiency and contribute to economic viability of biofuel industry.

The effectiveness of both radio frequency alkaline and steam explosion pretreatments at low severity factor were further evaluated by pelletizing the pretreated and non-pretreated barley straw samples in a single pelleting unit. The following quality parameters: pellet density, tensile strength, durability, and dimensional stability of the pellets were determined. It is very important to choose the right levels of process and material variables during pretreatment and densification processes, so as to produce biomass pellets with high quality characteristics, that can withstand various forces/agitation during transportation, handling, storage, and increase its higher heating value per unit volume, improve the flowability of biomass products, decrease the porosity, spillage and wind loss, above all improve the economical viability and its natural binding characteristics. The disruption and deconstruction resulting from the interaction between NaOH solution and the biomass matrix in the presence of RF heating assisted in improving the binding characteristics. The swelling pores created on the biomass matrix during the RF alkaline pretreatment process, subsequently made the binder (lignin) easily accessible during the densification process. The higher the temperature and retention time, the higher the durability, tensile strength, and fracture load of the pellets produced from SE pretreated samples. High temperature and long retention time helped in the local melting of some constituents (such as lignin), which subsequently enhanced the production of pellets with good physical characteristics. These quality characteristics (density, tensile strength, durability, and dimensional stability) can be used by the biofuel industry (pellets manufacturers) to perform relative comparison of quality of pellets produced from any kind of agricultural biomass.

Figure 8.1 shows the summary of the overall step by step procedure taking in the course of this Ph.D. project. Biomass barley straw bale was received and size reduction was performed to increase the surface area. The physical characteristics (such as particle, bulk, and solid densities) of the ground straw were conducted. Thereafter, the sample was subjected to RF or SE pretreatment to assist in breaking down the lignified biomass matrix and exposing the energy potentials (cellulose and hemicellulose) to any external reaction. The pretreated sample was further dried. The effects of the pretreatment were evaluated via chemical compositional analysis by using the wet chemical traditional approach (National Renewable Energy Laboratory standard-NREL) and Fourier transform infrared photoacoustic spectroscopy method.

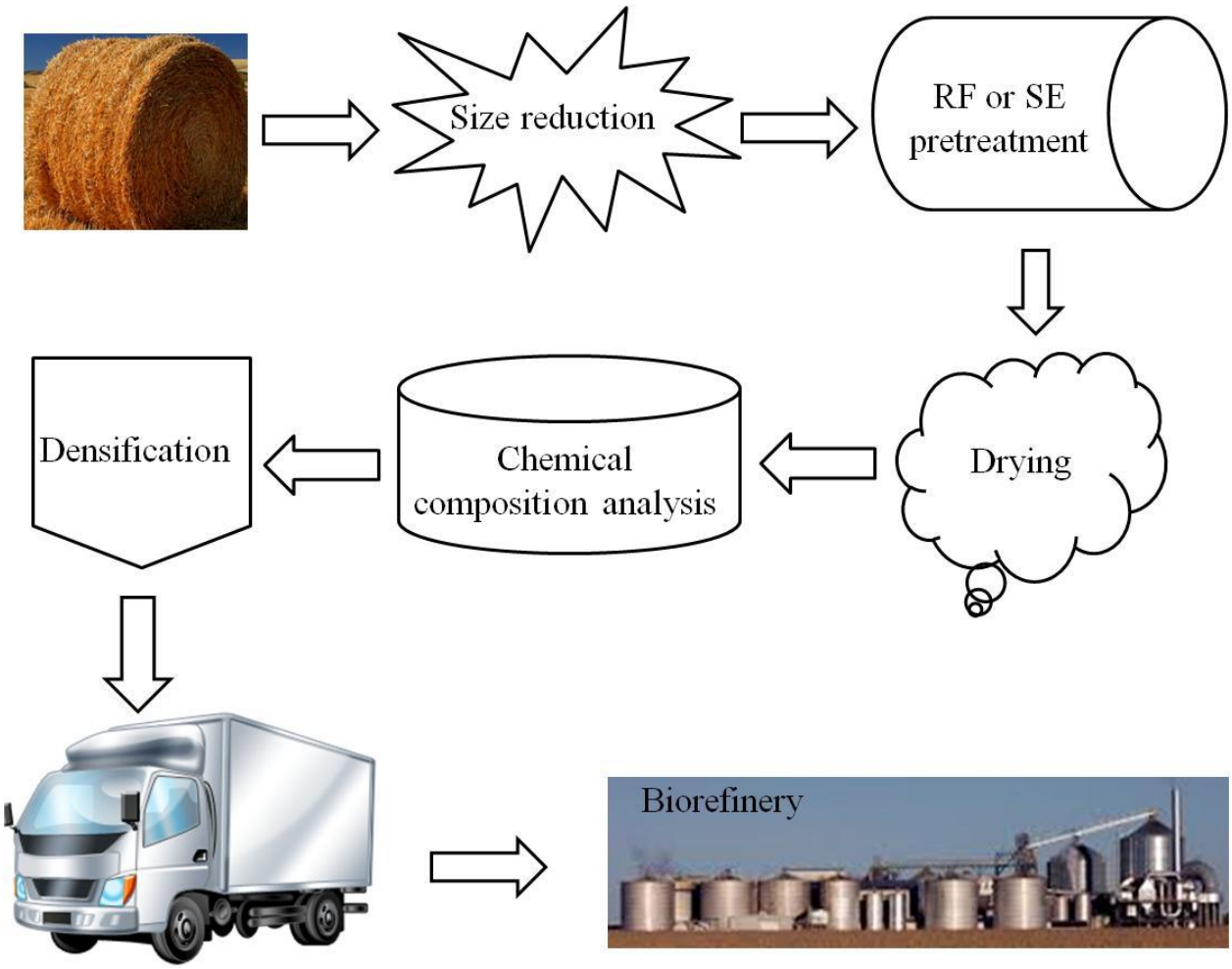


Figure 8.1 Process flow diagram of biomass barley straw, from pre-processing to biorefinery.

The pretreated sample was further densified into pellets for two reasons: 1) to further evaluate the effects of the pretreatments through the physical quality parameters (density, tensile strength, durability rating, and dimensional stability) of the produced pellets. 2) to improve storage, handling, and costs of transportation of the feedstocks to the biorefinery for processing. There might be situations where the biomass production location will be thousands of kilometers from the biorefinery (e.g. from coast to coast within Canadian or from Canada to Europe or Japan). In such cases, the biomass needs to be densified into pellets before transportation. To make good pellets requires initial breaking down and release of the natural binding components (lignin) of the biomass (pretreatment), which will also improve the enzymatic hydrolysis stage of the process. From this investigation, densification seems to act as another form of pretreatment.

There was a potential increase in reducing simple sugar of pelletized biomass. However, further study is required to confirm this finding. Enzymatic hydrolysis or saccharification is usually done within the biorefinery preceding fermentation. The hydrolysis process help to break the complex carbohydrates/polysaccharides (cellulose and hemicellulose) to simple reducing sugars (glucose, xylose, galactose, mannose, and arabinnose), which are used during the fermentation stage.

8.2 Achievement of Research Objectives and Contribution to Knowledge Advancement

The research objectives listed in Chapter 1 (Section 1.10) have been achieved over the course of the Ph.D. project. The research objectives are listed below from 1 to 6, while the thesis chapters in which the thesis research objectives are achieved are placed in brackets.

The main objective of this research is to investigate the pretreatment of lignocellulosic biomass (barley straw) to improve the accessibility of the energy potentials (cellulose and hemicellulose) and enhance densification process (Chapters 3-7).

The following specific objectives have been defined and achieved for this present research work:

1. to explore the potentials of radio frequency (RF) heating as a pretreatment method for the deconstruction and disaggregation of lignocellulosic biomass (Chapter 3)
2. to predict the degree of accessibility and digestibility of cellulose and hemicellulose for the subsequent stage of enzymatic hydrolysis/saccharification (Chapter 3 and 4)
3. to assess the operating conditions for steam explosion (SE) treatment of biomass straw at low severity factor and conditions with minimal production of inhibitors (furfural) (Chapter 4)
4. to validate and evaluate the readily available enzyme digestible glucose (cellulose) in the RF-alkaline and SE pretreated and non-treated samples (Chapter 5)

5. to study and rapidly estimate the relative chemical composition of lignin, cellulosic and hemicellulosic polymers in pretreated and non-treated lignocellulosic barley straw using Fourier Transform Infrared-Photoacoustic Spectroscopy, and also to develop an easy and non-laborious approach for the quantitative measurement of lignocellulosic biomass chemical compositions (Chapter 6)

6. to investigate the effects of RF-alkaline and SE pretreatments on the physical characteristics (density, tensile strength, durability, and dimensional stability) of pelletized barley straw, which could be used as feedstock for the production of bio-energy via thermochemical process (Chapter 7).

Chapter 9

9. Summary, Conclusions, and Recommendations

This chapter focuses on the overall general summary and conclusions of this project findings. It also detailed the projected recommendations for future studies.

9.1 Summary

To effectively make the renewable industry economically feasible and attract investors into the biofuel sector, it is important to consider the optimization of the production process. It is necessary to properly investigate the pretreatment of lignocellulosic biomass to improve the accessibility and digestibility of the energy potentials (cellulose and hemicellulose) and also enhance densification process. Pretreatment of biomass before subjecting it to enzymatic stage of the process enhances the penetration of enzymatic and microbiological activities into the cell-wall fine structure of lignocellulosic biomass. Subsequently, it assists in the separation of the structural linkages between lignin and the complex carbohydrates (cellulose and hemicellulose), increases the porosity of lignocellulosic biomass, and results to an increase in internal surface area.

Material and operating variables needed for the optimal dielectric properties (dielectric constant, dielectric loss factor, and power penetration depth) were fully investigated. Preliminary studies revealed that NaOH concentration, biomass screen size, soaking time, and the ratio of biomass to NaOH solution have significant effects on the dielectric properties of radio frequency (RF) biomass pretreatment process. Preliminary RF alkaline pretreatment was also conducted to fully optimize the process. During this optimization process, it was discovered that there was no significant difference between the acid soluble lignin moieties and acid insoluble lignin obtained from RF alkaline pretreated biomass ground using hammer mill at 0.8 and 1.6 mm screen size ;

as such, 1.6 mm screen size was adopted for this current investigation. This minimises energy use in particle size reduction. These optimized parameters and levels were subsequently applied to the actual RF alkaline pretreatment technique. RF heating generates uniform heat distribution with no local hot spots or thermal runaway. The use of NaOH solution and the ratio of biomass to NaOH solution played a more important role than temperature during the RF alkaline pretreatment. All ratios of biomass to NaOH solution that was tested had the following effects on the biomass: more lignin was structurally disrupted and released; lower acid insoluble lignin and higher total acid soluble lignin moieties were produced; the accessibility and digestibility of the cellulose and hemicellulose was increased, however, it leads to greater cellulose and hemicellulose degradation; the ash content was increased; and required longer pretreatment time. This implies that during the RF alkaline pretreatment, there is a trade-off between the breakdown of lignin bonds and carbohydrate degradation. It is recommended that RF with higher power level should be used to verify the longer pretreatment time observed in this study.

Steam explosion (SE) resulted in the breakdown of biomass matrix with increase in acid soluble lignin. However, there was a considerable thermal degradation of cellulose and hemicellulose with increase in acid insoluble lignin content. The high degradation of the hemicellulose can be accounted for by its amorphous nature which is easily disrupted by external influences unlike the well-arranged crystalline cellulose. The high initial moisture content of ground barley straw has buffer effect by lessening the effect of harsher steam pretreatment conditions. The severity factor increased correspondingly with increasing temperature and retention time. The carbon content of the solid steam exploded product increased at higher temperature and longer residence time, while the hydrogen and oxygen content decreased, and the higher heating value (HHV) increased.

The RF alkaline and SE treatment combinations that resulted to optimum yield of acid soluble lignin moieties, cellulose, and hemicellulose were selected and then enzymatically digested with a combined mixture of cellulase and β-glucosidase enzymes. The glucose in the hydrolyzed samples was subsequently quantified. The results obtained confirmed the effectiveness of the pretreatment processes. The data depicts that RF alkaline and SE pretreatment techniques create

reaction sites for enzymatic hydrolysis with a corresponding higher glucose yield relative to the non-treated sample. RF alkaline technique is more efficient than the SE. The average available percentage glucose yield that was released during the enzymatic hydrolysis for bioethanol production ranged from 78 to 96% for radio frequency alkaline pretreated samples and 30 to 50% for the steam explosion pretreated samples, depending on the treatment combination. While the non-treated sample has available average percentage glucose yields of just below 12%. The pretreated samples required low enzyme loading with a corresponding higher glucose yield unlike the non-treated sample. The difference between the glucose yield from RF and SE pretreatment can be accounted for by considering the initial carbonization that resulted to the severe degradation of the cellulose and hemicellulose during the SE process. The yield in RF alkaline pretreatment was better correlated with the ratio of biomass to NaOH solution followed by the RF temperature. Based on the digestible glucose yield, the most favorable pretreatment condition from the RF alkaline technique is ratio 1:6 (biomass to NaOH solution), 70^{o}C, 20 min retention time, 1% NaOH, with a hammer screen size of 1.6 mm. While the optimum condition for the SE pretreatment was identified to be at 160^{o}C, 30% moisture content, and 10 min retention time with a hammer screen size of 1.6 mm. Therefore, it can be concluded that to improve the yield of lignocellulosic biomass for bioethanol production, it is necessary to perform pretreatment prior to the enzymatic hydrolysis step.

In light of the need for rapid, easy and cost effective quantification of lignocellulosic biomass components (cellulose, hemicellulose, and lignin), the Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) was considered in determining the effects of various pretreatments (such as RF and SE) on lignocellulosic biomass feedstock for the biofuel industry. A model was developed to quantitatively predict lignocellulosic biomass components for both RF alkaline, SE pretreated and non-treated barley straw grind using FTIR spectroscopy. This model can be easily extended for any form of lignocellulosic biomass. This implies that the photoacoustic infrared spectra can be rapidly used for biofuel feedstock identification and analysis of the chemical compositions of lignocellulosic biomass before it is processed.

The effects of both RF and SE pretreatment methods were further evaluated by pelletizing the pretreated and non-pretreated barley straw samples in a single pelleting unit. The pellet density, tensile strength, durability, and dimensional stability of the pellets were determined. Radio frequency-alkaline pretreatment with the use of 1% NaOH solution and a ratio of biomass:NaOH solution of 1:8 has significant effect ($P<0.05$) on the breakdown of the lignified matrix, resulting in pellets with superior physical quality characteristics. The steam exploded samples pretreated at higher temperatures (180ºC) and retention time of 10 min resulted into pellets with good physical qualities. Overall, the pellets produced from the RF-alkaline pretreated samples have better physical quality properties (>15%) relative to that of the pellets from the SE pretreatment.

9.2 Project Conclusions

The following conclusions are made based on the experiments and analysis conducted in the course of this research:

1. The potential applicability of radio frequency (RF) alkaline heating as a pretreatment method for the deconstruction and disaggregation of lignocellulosic biomass has been explored. The RF alkaline pretreatment has the following effects on the biomass.

- Lignin matrix was structurally disrupted and released; lower acid insoluble lignin and higher total acid soluble lignin moieties were produced; accessibility and digestibility of the cellulose and hemicellulose increased.
- However, it leads to cellulose and hemicellulose degradation; increase in the ash content; and requires longer pretreatment time. This implies that during the RF alkaline pretreatment, there is a trade-off between the breakdown of lignin bonds and carbohydrate degradation.
- Alkaline (NaOH) solution increased the ash content of pretreated biomass.

2. The degree of accessibility and digestibility of cellulose and hemicellulose for the subsequent stage of enzymatic hydrolysis/saccharification was predicted to increase by 64% and 33% for the RF alkaline and SE pretreated sample, respectively. This will assist in reducing the costs and amount of enzymes required for the next stage of process (enzymatic hydrolysis) by

about 64% and 33% for the RF alkaline and SE pretreated sample, respectively, compared to the non-treated biomass.

3. The severity factor of SE pretreatment increased with increasing temperature and time. Moisture content of biomass acts as a buffer to dampen the severity of the pretreatment condition, and subsequently moderate the formation of inhibitors such as furfural.

- Degradation of cellulose hemicellulose occurred during SE pretreatment. Hemicellulose degrades more than cellulose due to the amorphous nature of hemicellulose.
- The acid insoluble lignin increased as the temperature increased resulting from the degraded cellulose and hemicellulose which appears as residue in the pretreated biomass.
- SE increased the carbon content and higher heating value of biomass.

4. RF alkaline pretreatment is more efficient than the SE. The average available percentage glucose yield that was released during the enzymatic hydrolysis for bioethanol production ranged from 78 to 96% for RF-alkaline pretreated samples and 30 to 50% for the SE pretreated samples, depending on the treatment combination.

5. Fourier transform infrared (FTIR)-photoacoustic spectroscopy was successfully applied, and subsequently, regression models were developed to rapidly estimate the lignocellulosic biomass content. This procedure could be easily adopted by the biofuel industry and extended to any form of lignocellulosic biomass using FTIR spectroscopy.

6. RF-alkaline and SE pretreatments improve the physical quality characteristics (density, tensile strength, durability, and dimensional stability) of pelletized barley straw, which could be used as feedstock for the production of bio-energy via thermochemical process.

9.3 Project Recommendations

The following recommendations were made for future investigations.

- The long heating time observed in this investigation was as a result of the low power level (1.5 kW) of the laboratory RF machine and the protruding space on the lid of the blown glass reactor used, which created a gap/space between the top level of the prepared

biomass-NaOH solution mixture and the lid of the reactor. Space decreases dramatically the electric field strength generated between the RF electrodes as air acts as resistance. As such, it took more time to heat the mixture. Therefore, for future and scale-up purposes, to increase the heating rate, RF at higher power levels should be used to verify the longer pretreatment time observed in this study. The available RF power level for commercialisation is now up to several hundred kW.

- It is recommended also that the reactor needs to be redesigned so as to bring the distance between the surface of the biomass-NaOH solution mixture and the male (top) electrode to a minimum.
- RF assisted-alkaline pretreatment technique represents an easy to set-up and potentially affordable route for the biofuel industry, but this requires further energy analysis and economic validation.
- Online monitoring system of the chemical compositions and breaking down of the lignified matrix of biomass during the pretreatment process may be necessary to avoid further degradation of the energy potentials (cellulose and hemicellulose). The target should be to permit the breaking down or disruption of only the lignified layer of the biomass and stop the process before the reaction process crops into the polysaccharides layer.
- Densification of biomass may be another form of pretreatment process. The densified biomass from RF-alkaline and SE pretreated samples were observed to have higher available average percentage glucose yield that was released during the enzymatic hydrolysis. This may be due to the compaction/compression of the biomass at the preset densification temperature, which may have caused further deconstruction/disruption of the biomass matrix. From this investigation, it is being hypothesized that compression/densification/pressing alters the biomass physical and chemical structures (and further melt and caused re-distribution of alkaline within the cell cavities of the RF pretreated biomass), leading to further breaking down of the biomass bonds during the densification process. Consequently, higher hydrolysis yield of soluble sugar (glucose) resulted. However, the information/data obtained from this current investigation is not

sufficient to conclude this trend. As such, further investigation is required in future to collect enough data to ascertain the results of this preliminary finding/investigation.

- Further enzymatic saccharification should also be conducted to ascertain the effects of RF and SE pretreatments on other simple sugars such as xylose, galactose, mannose, and arabinnose.
- The severity parameters (temperature, residence time, and biomass moisture content) which are keys to the optimization and design of novel steam explosion pretreatment were investigated in the course of this project. This phase of the experiment was performed at the Clean Energy Research Center, University of British of Columbia, Vancouver, BC. As always, when using experimental set up that is stationed offshore, the time available limits the number of experimental trials that can be conducted. Therefore, the construction of the experimental set up of steam explosion pretreatment facility within this department is highly recommended to give room for several experimental trials and also from an economic point of view.
- Further studies may be required to rapidly estimate and correlate color and higher heating value of steam exploded biomass at various severity factors. The color of steam pretreated samples can be used as an indicator of carbonization during steam explosion pretreatment, as samples tend to darken with increased carbonization.

Chapter 10

10. References

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Appendix A

A. Material and Operating Variables Affecting the Dielectric Properties of Lignocellulosic Barley Straw

A similar version of this chapter has been presented at the 2nd Conference of Agricultural Biorefinery Innovation Network for Green Energy, Fuels and Chemicals (ABIN):

- Iroba, K.L. and L.G. Tabil. 2011. Material and operating variables affecting the dielectric properties of lignocellulosic barley straw. 2nd Conference of Agricultural Biorefinery Innovation Network for Green Energy, Fuels and Chemicals (ABIN), Ivey Spencer Leadership Centre London Ontario, Canada. January 9-11, 2011.

A similar version of this chapter has also been presented and published in the International conference of Canadian Society of Biological Engineers and American Society of agricultural and Biological Engineers:

- Iroba, K.L. and L.G. Tabil. 2010. Dielectric Properties of Lignocellulosic Biomass Barley Straw Relevant to Radio-frequency Pretreatment Process. CSBE/ASABE North Central Inter-sectional Conference, Saskatoon, SK, Paper No. MBSK10-500, Oct. 7-9.

A.1 Abstract

Dielectric properties of lignocellulosic biomass barley straw and sodium hydroxide/water solution relevant to radio frequency pretreatment processes were measured over frequency of 10 to 30 MHz using the precision LCR meter and liquid test fixture. Effects of the process temperature, particle moisture content, sodium hydroxide concentration, particle size, soaking

time, and ratio of biomass to alkaline/water solution on dielectric constant, dielectric loss factor, and power penetration depth were investigated. Dielectric constant and dielectric loss factor of lignocellulosic biomass-alkaline mixture decreased with increasing frequency. Dielectric constant and dielectric loss factor increases with increasing NaOH concentration, particle size, and soaking time. The dielectric constant and dielectric loss factor increased with increasing ratio until it reached maximum and decreased at the critical ratio R_c. The critical ratio depends on the operating temperature. The power penetration depth had an inverse relationship with all the material and process variables. Penetration depth decreased as any of the material or process variables increases. Regression equations were developed to relate the dielectric properties to temperature, particle size, NaOH concentration, soaking time, and ratio of biomass to alkaline/water solution.

A.2 Introduction

The environmental pollution resulting from the use of fossil fuel has in recent time attracted worldwide attention. The use of renewable and carbon neutral type of energy has been considered to be the best option to avoid further degradation of our environment and the climate at large (Fiona et al. 2007). Lignocellulosic biomass straw is one of the alternative sources that can be used to produce cellulosic ethanol (Demirbas et al. 2009). However, a large amount of enzyme is required to convert the polysaccharide (cellulose and hemicellulose) into fermentable sugars due to the lignin cross linking binding nature and the high level of crystallinity of the cellulose (Fan et al. 2006b; Sun and Cheng 2002; Söderström et al. 2003). Therefore, this necessitates the deconstruction of the feedstock via pretreatment by application of heat and other compounds (such as acids and alkaline) (Mohammad and Karimi 2008). This will subsequently enhance the digestibility and accessibility of the complex carbohydrates during enzymatic hydrolysis. Most of these pretreatment methods require high temperature, which is usually achieved by convection- or conduction mode of heat transfer. Higher efficiency of the process is obtained by uniformly heating up the sample and kept at such temperature during the whole process. Radio frequency (RF) and microwave (MW) heating can be considered as alternative methods for the pretreatment of lignocellulosic biomass. In RF and MW, the heating is based on volumetric heat generation, which is rapid (Piyasena et al. 2003). This method of heating does

not necessarily require large quantity of solvent in the material, and thus reduce the reactor volume and implies low capital cost. The uniformity of the electric field strength in RF helps to avoid thermal degradation or formation of local hot spots in some locations of the reactor (Piyasena et al. 2003; Balakrishnan et al. 2004). RF has higher wavelength than MW due to the lower frequency used in RF. Therefore, higher power penetration depth in RF provides efficient heating for large RF reactors on an industrial scale. These are the inherent advantages of RF over MW and conventional heating (Piyasena et al. 2003; Balakrishnan et al. 2004; Maurizio et al. 2004; Ramaswamy and Tang 2008). To avoid interference problems with telecommunication devices and radar transmissions, selected frequency bands are only used for industrial, scientific, and medical (ISM) applications (Schubert and Regier 2005). RF frequencies (13.56 27.12, and 40.68 MHz) and MW frequencies (896, 915, 2375, and 2450 MHz) are allocated within the electromagnetic spectrum in different countries for the ISM uses (Schubert and Regier 2005).

RF has been successfully applied on drying (Balakrishnan et al. 2004), on thermal therapy (Maurizio et al. 2004), and application of RF heating has been reported in other research fields such as food processing (blanching, tempering, pasteurization, sterilization) and medicine (Piyasena et al. 2003). In recent time, research has demonstrated that RF can be used for the extraction of podophyllotoxin from rhizomes of *P. peltatum* (Izadifar and Baik 2009). RF heating has been used as a treatment method to inhibit the growth of fungus in wood samples in an industrial 40 kW dielectric oven at temperatures between 60 and 70°C for 2 min (Tubajika et al. 2007). As reported, the results showed that RF radiation can completely inhibit fungus by 98-100%. However, no study has been reported on the use of RF heating as a pretreatment method on lignocellulosic biomass barley straws. Radio frequency heating system is associated with the distribution of electrical field/energy which is created by an RF generator (Ryynanen 1995). The RF reactor is placed between two charged conductive plates (electrodes or capacitors), which are charged by the generator. The four fundamental mechanisms that contribute to the dielectric loss factor of a material are atomic polarization (occurs in atoms), electronic polarization (occurs in atoms), orientation or dipole polarization (water), and ionic conductivity (Ryynanen 1995). Ryynanen (1995) reported that ionic conductivity is dependent on temperature. The dominant loss mechanisms (dielectric loss factor) at RF and MW frequencies relevant to industrial

dielectric heating of materials or foods are ionic conductivity and dipole polarization/rotation (Ryynanen 1995; Schubert and Regier 2005).

When samples are subjected to electric field, the ions in the solvent/particles tend to move at fast rate and migrate towards the opposite charged electrodes (Ryynanen 1995; Schubert and Regier, 2005). The resulting collision/friction/flip flop rotation causes increase in temperature. The polar structure of water is separated by a small gap (a dipole) (Mohsenin 1984). Such structure is the same as a pair of equal and opposite charges. The polar molecule (water molecule) tends to align itself with the polarity of the electromagnetic field (Mohsenin 1984). As a result, there are significant ionic and molecular frictions leading to volumetric heat generation within the sample (Izadifar and Baik 2009). Therefore, dielectric properties of lignocellulosic biomass straw used in RF pretreatment process play an important role in achieving the optimal and desired performance of the delignification process. As a result, a basic understanding of dielectric properties of biomass straw is needed.

A.2.1 Dielectric Properties of Materials

Dielectric properties of materials are the vital characteristics that mostly affect the efficiency of electromagnetic (EM) energy within the materials (Schubert and Regier 2005). Dielectric properties are necessary physical properties connected with RF and MW heating (Schubert and Regier 2005).

The dielectric properties of a material can be measured with Agilent 16452A precision LCR meter (Agilent Technologies, Palo Alto, CA) using "Capacitive Method" by measuring the capacitance of a material/medium that is sandwiched between parallel electrodes. Capacitance, C of a material is a measure of the ability of such material to hold charge if a voltage is applied across it (Agilent Technologies, Palo Alto, CA), it can be mathematically represented as:

$$C = \varepsilon\left(\frac{A}{t}\right) \tag{A.1}$$

Where *A* is the surface area of the electrode, *t* is the separation gap/distance between the two electrodes/conductive plates, ε is permittivity (which is a measure of the ability of a material/medium to be polarized by an electric field). When the dielectric used is vacuum, then the capacitance:

$$C_0 = \varepsilon_0\left(\frac{A}{t}\right) \tag{A.2}$$

Dielectric constant represents the ability of the sample or material to store electrical energy or the energy value of the sample or material when subjected to electromagnetic (EM) field (Schubert and Regier 2005). The dielectric constant is also known as the relative permittivity of the material (Schubert and Regier 2005). The dielectric constant of a material is the ratio of its permittivity ε to the permittivity of vacuum ε_0 ($\varepsilon' = \varepsilon/\varepsilon_0$) therefore, it can be represented as a relative term ε'_r. As such, the dielectric constant of vacuum is 1 (Schubert and Regier 2005; Mohsenin 1984). The dielectric constant is a dimensionless quantity because it is a ratio of two similar quantities.

The dielectric loss factor reflects the ability of the material to dissipate the electrical energy and convert electrical energy into thermal energy within the material (Venkatesh and Raghavan 2004; Schubert and Regier 2005; Piyasena et al. 2003). This parameter represents how well the material can be penetrated by an electrical field and how it dissipates the electrical energy. The attenuation power is determined by two important quantities, the tangent of dielectric loss angle and the dielectric constant (Schubert and Regier 2005). The tangent of dielectric loss angle is dependent on the relaxation time τ, and the periodic time, 1/f (f is frequency). The relaxation time is defined as the time for dipoles in a material to return to their random orientation after the removal of the electric field (Piyasena et al. 2003).

Therefore, it is important to have a good understanding of the dielectric properties of biomass barley straw so as to meet the desired optimal process condition requirements for the RF pretreatment process. The objectives of this study were to investigate the influence of frequency, particle moisture content, operating temperature, sodium hydroxide concentration (NaOH), soaking time, biomass particle size, and ratio of biomass to water/NaOH solution on the dielectric properties of lignocellulosic biomass barley straw. The obtained data and information

can be used for design and optimization of laboratory and industrial RF pretreatment process of biomass in the future.

A.3 Materials and Methods

A.3.1 Sample Procurement and Preparation

Lignocellulosic biomass barley straw was obtained in October, 2009 from RAW Ag Ventures Limited (Maymont, SK). The straw was ground using the laboratory grinder (Serial no. GM13688; Glen Mills Inc., Maywood, NJ). A hammer mill fitted with screen sizes of 0.8 and 1.6 mm was used to grind the biomass straw. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation and to provide flow of the biomass straw in and out of the hammer mill. The initial moisture content of the straw was 8.8% (d.b.). The moisture content was measured based on ASABE standard method S358.2 DEC1988 (R2008). In this study, water and sodium hydroxide were used as solvent, and the samples were prepared by using the following ratios of biomass to water or biomass to sodium hydroxide solution as shown in Table A.1.

Table A.1. Ratios of biomass to water/NaOH, equivalent masses and corresponding moisture content

Ratio of biomass to water or NaOH solution	Equivalent mass of biomass (g)	Equivalent mass of water or NaOH (g)	Corresponding moisture content % (w.b.)
1:9	0.5	4.5	91
1:8	0.5	4.0	90
1:7	0.5	3.5	89
1:6	0.5	3.0	87
1:5	0.5	2.5	85
1:4	0.5	2.0	82

A.3.2 Physical Characteristics of Barley Straw

The particle density of the samples was measured using the gas multi-pycnometer (model No. MVP-2, QuantaChrome, Boynton Beach, FL) by calculating the displaced volume of nitrogen gas by a known mass of material. Three replicate tests were performed on each sample. The bulk density of the samples was determined by passing the material through a funnel which was

placed above a standard 0.5 liter steel cup (SWA 951, Superior Scale Co. Ltd., Winnipeg, MB). Blockages in the funnel during biomass straw flow were cleared using a thin steel rod. After filling the cup, the excess was removed by passing a steel roller in a zig-zag manner over the top of the cup. The mass contained in the cup was then determined. Bulk density was calculated by dividing the mass of material within the cup by the volume of the cup. Three replicates were performed for each sample. The inter-particle porosity ($\varnothing_{ip}$) of the ground samples were computed from the particle and bulk densities (Table A.2) ρ_p and ρ_b, respectively, using equation A.3.

$$\phi_{ip} = 1 - \frac{\rho_b}{\rho_p} \tag{A.3}$$

Table A.2. Physical characteristics of barley straw

Physical characteristics	Hammer mill screen size	
	0.8 mm	1.6 mm
Particle density (kg m^{-3})	1272.72 (0.59)	1126.57 (2.14)
Bulk density (kg m^{-3})	124.75 (0.19)	98.35 (0.76)
Inter-particle porosity (%)	90 (0.00)	91 (0.00)

A.3.3 Dielectric Properties Measurement

Agilent 16452A Liquid test fixture and Agilent 4285A precision LCR meter (Agilent Technologies, Palo Alto, CA) were used to measure the dielectric properties of water, NaOH solution, biomass barley straw and water mixture, and biomass barley straw and NaOH solution mixture. The liquid test fixture and the precision LCR meter were connected using SMA-BNC adaptor and 1 m Agilent 16452-61601 test leads cables (Agilent Technologies, Palo Alto, CA) that measure temperature ranging from -20 to +125^{o}C. The operating frequency of the precision LCR meter ranges from 20 Hz to 30 MHz. The liquid test fixture was calibrated prior to the analysis based on Agilent technology standard. The cable length correction, short residual, and short compensation associated with the liquid test fixture were also performed. The liquid test fixture contains two parallel electrodes that are separated by a spacer of 2 mm thickness, which corresponds to the electrode gap. This setup measures the electrical properties of the sample (the capacitance and resistance), which are subsequently used for the calculation of the dielectric properties using equations A.4, A.5, and A.6 below. Capacitance (C) is a measure of the ability

of a material/medium to hold charge when a voltage is applied across it (C = Q/V) (Agilent Technologies, Palo Alto, CA). The unit of measurement for capacitance is the farad (coulomb per volt). Capacitance is best modeled by a dielectric layer that's sandwiched between two parallel electrodes/conductive plates (Agilent Technologies, Palo Alto, CA).

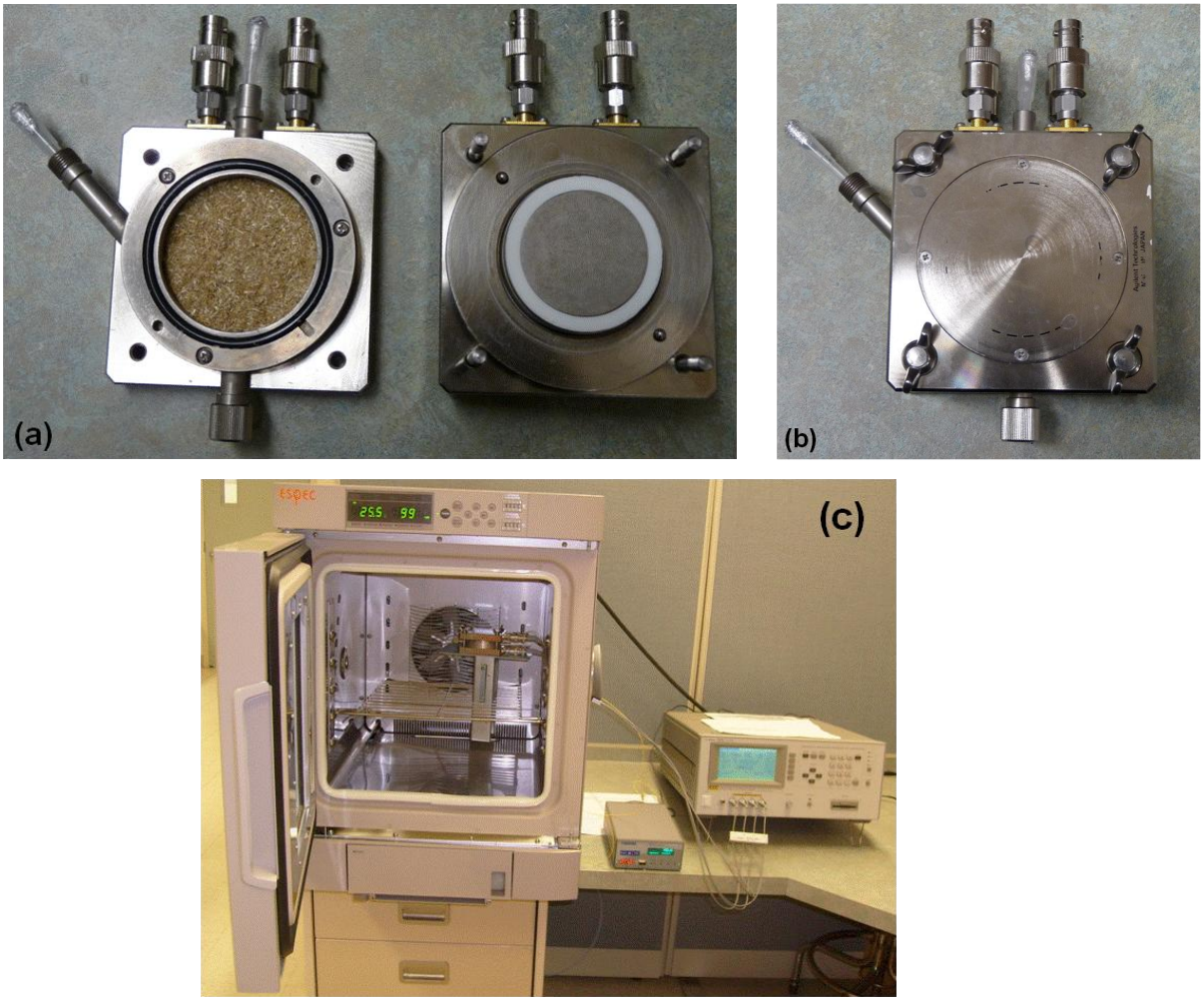


Figure A.1 Procedure involved in the preparation of biomass straw and water/NaOH solution in the 16452A liquid test fixture for the measurement of dielectric properties. Sample loaded into the lower electrode assembly of the fixture a); fixture completely tightened and the liquid inlet and air outlet firmly sealed b); the heating chamber c).

Figure A.1 depicts the steps and procedure involved in the measurement of the dielectric properties of lignocellulosic biomass barley straw with water or NaOH solution using the liquid

test fixture. Before and after each test, the liquid test fixture was cleaned with water and dried thoroughly. The required biomass straw samples were weighed with a weighing balance (Adventurer Pro AV812, Ohaus Corporation, Pine Brook, NJ) with accuracy of $\pm 0.01g$, and loaded into the lower electrode assembly of the fixture (Figure A.1a). The water or NaOH solution was measured using a syringe and added to the biomass in the fixture. To avoid any compositional loss, this was prepared by careful mixing of the sample and water or NaOH solution in the lower electrode assembly liquid test fixture (based on the above mentioned ratios). The top of the mixture was carefully smoothening to avoid any kind of sparks/bubbles which may interfere with the accuracy of the measurement. Thereafter, the spacer was placed on the lower electrode assembly followed by fixing the high electrode assembly which is the second part of the liquid test fixture. The fixture was completely tightened and the liquid inlet and air outlet were firmly sealed using two stoppers to avoid external interference of the measurement (Figure A.1b). The prepared samples were kept at room temperature for 30 minutes to equilibrate before measurement. The water used for the experiment was tap water, because tap water has good conductivity and contains dissolved ions and solutes required for the ionic conductivity during the RF pretreatment process.

Based on the factors that may affect the dielectric properties of materials relevant to the radio frequency heating, the following factors were considered in the measurement of the dielectric properties of the lignocellulosic biomass barley straw: frequency, temperature, particle moisture content, particle size, soaking time, NaOH concentration, and ratio of biomass to water/NaOH solution. The electrical properties (the capacitance and resistance) of the prepared samples were measured based on the different levels in Table A.3

Table A.3. Material and operating variables investigated

Parameters	**Levels**
Frequency	10, 13.56, 15, 17, 19, 21, 23, 25, 27.12, and 30 MHz
Temperature	40, 50, 60, 70, 80, 90, and 100°C
Particle size	0.8 and 1.6 mm
Concentration	0, 0.5, and 1 %
Soaking time	0 & 30 min
Ratio of biomass to NaOH solution	1:4, 1:6, 1:7, 1:8, 1:9, and 1:10

Alkaline (NaOH) is used in this study because it has a major effect on the delignification of lignocellulosic biomass during the pretreatment process. Alkaline pretreatment enhances the removal of acetyl group (organic group of acetic acid, with a methyl group single-bonded to a carbonyl (CH_3CO-) and different types of uronic acid substitutions on hemicellulose. These compounds lower the degree of enzymatic hydrolysis of cellulose and hemicellulose (Chang and Holtzapple 2000). Swelling occurs in biomass that is treated with dilute alkaline solution. This subsequently, increases the internal surface area which effectively enhances accessibility and digestibility of lignocellulosic biomass, decreasing the crystallinity and the degree of polymerization in cellulose, and disconnecting the structural linkages between lignin and the complex carbohydrates (cellulose and hemicellulose) (Mosier et al. 2005; Sun and Cheng 2002; Wang et al. 2008).

The two levels of NaOH solution concentrations (0.5% and 1%) used in this study were prepared by dissolving 5 and 10 g of NaOH granules in 1 liter of water, w/v, respectively. To control the temperature of the prepared samples, a heating chamber (SH-641 with serial No. 92005408, ESPEC CORP. Japan) with accuracy of $\pm 0.1^{o}C$ was used. The fixture containing the prepared sample was placed horizontally (to avoid any phase separation) in the heating chamber using the fixture stand. The fixture was connected to the precision LCR meter with the 16452-61601 test leads via an opening on the wall of the heating chamber Figure A.1c. This opening was subsequently sealed with a foam stopper. The chamber was switched on and set to the preset temperature required for the measurement. After the chamber attained the desired temperature, the fixture was left to remain inside the chamber for about 27 min for the thermal equilibrium. The thermal equilibrium time was determined in the preliminary experiments with a Neoptix fiber optic temperature sensor (Neoptix, Inc.1415 Frank-Carrel, Quebec, QC). This time was adopted for all the experiments. Each of the experiment was done in duplicate.

A.3.4 Determination of the Dielectric Constant and Loss Factor

The dielectric constant ($\varepsilon_r^{'}$) and loss factor ($\varepsilon_r^{''}$) were computed from the capacitance, C_P (F) and resistance, R_p (Ω). The electrode dimensions were also included in the calculation using the following equations (Wang et. al 2005; Agilent technologies 2005):

$$\varepsilon_r^{'} = \frac{tC_P}{A\varepsilon_0} \tag{A.4}$$

$$\varepsilon_r^{''} = \frac{\sigma}{\omega\varepsilon_0} \tag{A.5}$$

Where, $\sigma = \frac{t}{AR_p}$, and (A.6)

$$\omega = 2\pi f\,, \tag{A.7}$$

Therefore,

$$\varepsilon_r^{''} = \frac{t}{2\pi f R_p \varepsilon_0 A} \tag{A.8}$$

t is the gap between the electrodes (for this experiment *t* = 0.002 m), *A* is the surface area of the electrode (0.00108 m^2), σ is conductivity, ω is angular frequency, and *f* is frequency (Hz). The diameter of the electrodes used is 0.037 m. The permittivity of vacuum (dielectric constant of vacuum), ε_0 can be calculated from the capacitance of the vacuum based on equation A.2 above, which can be transformed and approximately equal to air capacitance, C_0:

$$\varepsilon_0 = \frac{tC_0}{A} \tag{A.9}$$

A.3.5 Determination of Power Penetration Depth

This is one of the important dielectric processing parameters. The penetration depth is defined as the depth below a material's surface where the dissipated power is reduced to 1/e (Euler's number e ≈ 2.718) or 36.8% of its original power or transmitted value entering the surface (Venkatesh and Raghavan 2004; Schubert and Regier 2005). The 'value of the original power P_o' is defined as the power which has entered the material: the incident power (P_i) minus the reflected power (P_r). The penetration depth d_p in meters can be calculated from the dielectric constant and loss factor using (Von Hippel 1954; Schubert and Regier 2005):

$$d_p = \frac{c}{2\sqrt{2\pi f}\left\{\varepsilon_r'\left[\sqrt{1+\left(\varepsilon_r''/\varepsilon_r'\right)^2}-1\right]\right\}^{1/2}} \quad \text{(A.10)}$$

c is the speed of light ($3x10^8$ m/s in vacuum/free space).

The wavelength λ (m), frequency f (Hertz, 1/s), and velocity ν (3×10^8 ms^{-1}) of electromagnetic waves are related by:

$$\lambda = \frac{v}{f} \quad \text{(A.11)}$$

This implies that the wavelength and penetration depth into a material are inversely proportional to the frequency. Therefore, this accounts for the reason why RF (with lower frequency) has a higher penetration depth than MW with higher frequency. With relatively high dielectric constant and loss factors, moist materials do not permit deep penetration of the EM into the material (Buffer 1993; Guan et al. 2004; Schubert and Regier 2005). Penetration depth is an indicator of the relative uniform heating of materials in an EM field at a particular frequency.

A.3.6 Statistical Analysis

Experimental data were statistically analyzed via analysis of variance (ANOVA) using the multiple regression analysis. It was conducted at 5% significance level using SPSS (Superior Performing Statistical Software version 14 for Windows, 2005; IBM, Armonk, New York, NY) for dielectric constant and dielectric loss factor at frequency of 13.56 and 27.12 MHz.

A.4 Results and Discussion

Tables A.3 to A.6 illustrates the mean values of the dielectric constant, dielectric loss factor, and power penetration depth of alkaline-biomass mixture based on the tested variables. However, most of the results presented below are based on 27.12 MHz frequency, because this is the frequency at which the available laboratory RF equipment operates. Therefore, it is necessary to fully understand the dielectric behavior of lignocellulosic biomass straw at this frequency.

A.4.1 Factors Affecting Dielectric Properties

The dielectric properties of materials are influenced by the following factors: frequency, temperature, moisture content and chemical solution concentration, material compositions, ratio of biomass to solution, soaking time, and particle size.

Table A.4. Mean values of the dielectric constant, dielectric loss factor, and power penetration depth of alkaline-biomass mixed with 0% NaOH concentration, 0.8 mm particle size, 13.56 and 27.12 MHz, at six levels of temperature, and at ratio 1:8

Frequency (MHz)	Temperature (^{o}C)	Dielectric constant	Dielectric loss factor	Power penetration depth (m)
13.56	40	110.20 (2.48)	366.54 (12.05)	0.130 (0.002)
27.12	40	89.47 (1.84)	195.30 (6.43)	0.0890 (0.001)
13.56	50	115.11 (2.66)	419.74 (12.64)	0.122 (0.002)
27.12	50	92.57 (2.11)	224.06 (6.68)	0.083 (0.001)
13.56	60	118.55 (3.28)	467.61 (16.52)	0.115 (0.002)
27.12	60	94.54 (2.64)	249.75 (8.71)	0.079 (0.001)
13.56	70	118.28 (3.89)	500.41 (20.67)	0.111 (0.002)
27.12	70	93.93 (3.17)	267.05 (10.82)	0.076 (0.002)
13.56	80	109.71 (2.69)	509.66 (50.79)	0.110 (0.006)
27.12	80	86.71 (2.14)	258.78 (8.14)	0.077 (0.001)
13.56	90	93.91 (14.95)	414.53 (83.49)	0.123 (0.012)
27.12	90	73.89 (11.96)	221.76 (44.07)	0.084 (0.008)

Values in parenthesis = standard deviation

Table A.5. Mean values of the dielectric constant, dielectric loss factor, and power penetration depth of alkaline-biomass mixed with 0.5% NaOH concentration, 0.8 mm particle size, 13.56 and 27.12 MHz, at seven levels of temperature and at ratio 1:8

Frequency (MHz)	Temperature (^{o}C)	Dielectric constant	Dielectric loss factor	Power penetration depth (m)
13.56	40	140.07 (2.71)	965.47 (33.18)	0.080 (0.001)
27.12	40	108.10 (2.02)	502.95 (17.40)	0.056 (0.001)
13.56	50	148.30 (1.96)	1106.82 (35.51)	0.075 (0.001)
27.12	50	110.90 (1.37)	577.32 (18.51)	0.052 (0.001)
13.56	60	152.98 (2.86)	1232.68 (40.70)	0.071 (0.001)
27.12	60	110.28 (1.92)	642.90 (21.46)	0.049 (0.001)
13.56	70	152.37 (1.63)	1327.14 (34.28)	0.068 (0.001)
27.12	70	106.20 (0.81)	691.52 (18.06)	0.047 (0.001)
13.56	80	154.13 (0.06)	1422.60 (6.63)	0.066 (0.000)
27.12	80	103.97 (0.48)	741.32 (3.50)	0.046 (0.000)
13.56	90	154.71 (9.29)	1477.29 (12.15)	0.065 (0.003)
27.12	90	100.83 (5.19)	771.24 (63.58)	0.045 (0.002)
13.56	100	153.57 (22.85)	1516.40 (301.65)	0.064 (0.006)
27.12	100	97.12 (11.73)	792.37 (159.31)	0.045 (0.005)

Values in parenthesis = standard deviation

Table A.6 Mean values of the dielectric constant, dielectric loss factor, and power penetration depth of alkaline-biomass mixed with 1% NaOH concentration, 0.8 mm particle size, 13.56 and 27.12 MHz, at seven levels of temperature, and ratio of 1:8

Frequency (MHz)	Temperature (^{o}C)	Dielectric constant	Dielectric loss factor	Power penetration depth (m)
13.56	40	245.20 (1.92)	2608.11 (109.65)	0.049 (0.001)
27.12	40	136.32 (2.96)	1381.48 (58.10)	0.034 (0.001)
13.56	50	245.60 (2.09)	2865.47 (47.06)	0.047 (0.000)
27.12	50	124.16 (0.23)	1523.09 (21.63)	0.032 (0.000)
13.56	60	240.84 (0.14)	3070.67 (51.13)	0.045 (0.000)
27.12	60	114.14 (0.96)	1632.72 (23.37)	0.031 (0.000)
13.56	70	231.16 (4.24)	3193.14 (54.59)	0.044 (0.000)
27.12	70	101.96 (3.98)	1695.93 (25.86)	0.030 (0.000)
13.56	80	203.01 (5.00)	3120.16 (61.96)	0.045 (0.000)
27.12	80	91.59 (7.09)	1649.87 (30.81)	0.031 (0.000)
13.56	90	188.74 (16.39)	2940.98 (413.79)	0.046 (0.003)
27.12	90	90.29 (4.13)	1550.92 (222.34)	0.032 (0.002)
13.56	100	187.33 (14.09)	3084.56 (432.08)	0.045 (0.003)
27.12	100	86.48 (2.16)	1628.23 (232.72)	0.031 (0.002)

Values in parenthesis = standard deviation

Table A.7 Mean values of the dielectric constant, dielectric loss factor, and power penetration depth of alkaline-biomass mixed with 1% NaOH concentration, 1.6 mm particle size, 13.56 and 27.12 MHz, at seven levels of temperature, and at ratio of 1:8

Frequency (MHz)	Temperature (^{o}C)	Dielectric constant	Dielectric loss factor	Power penetration depth (m)
13.56	40	264.35 (7.51)	2334.44 (48.76)	0.052 (0.001)
27.12	40	136.28 (3.83)	1241.15 (25.28)	0.035 (0.000)
13.56	50	266.29 (5.79)	2550.12 (32.98)	0.049 (0.000)
27.12	50	128.10 (5.04)	1358.69 (15.71)	0.034 (0.000)
13.56	60	266.18 (9.15)	2712.28 (30.85)	0.048 (0.000)
27.12	60	121.83 (7.26)	1445.61 (14.66)	0.033 (0.000)
13.56	70	250.91 (7.63)	2721.38 (15.06)	0.048 (0.000)
27.12	70	112.33 (6.83)	1447.78 (5.68)	0.033 (0.000)
13.56	80	223.09 (0.06)	2685.98 (138.82)	0.048 (0.001)
27.12	80	101.88 (5.92)	1422.75 (78.28)	0.033 (0.001)
13.56	90	219.03 (3.07)	2703.68 (18.07)	0.048 (0.000)
27.12	90	97.33 (4.95)	1432.69 (12.59)	0.033 (0.000)
13.56	100	205.58 (17.24)	2570.91 (319.01)	0.049 (0.003)
27.12	100	92.57 (3.21)	1357.86 (168.47)	0.034 (0.002)

Values in parenthesis = standard deviation

A.4.2 Frequency Effects

Figure A.2 shows the variation of the mean values of dielectric constant (Figure A.2a) and dielectric loss factor (Figure A.2b) of tap water and 0.5% NaOH solution for frequencies between 10 to 30 MHz at 30^{o}C.

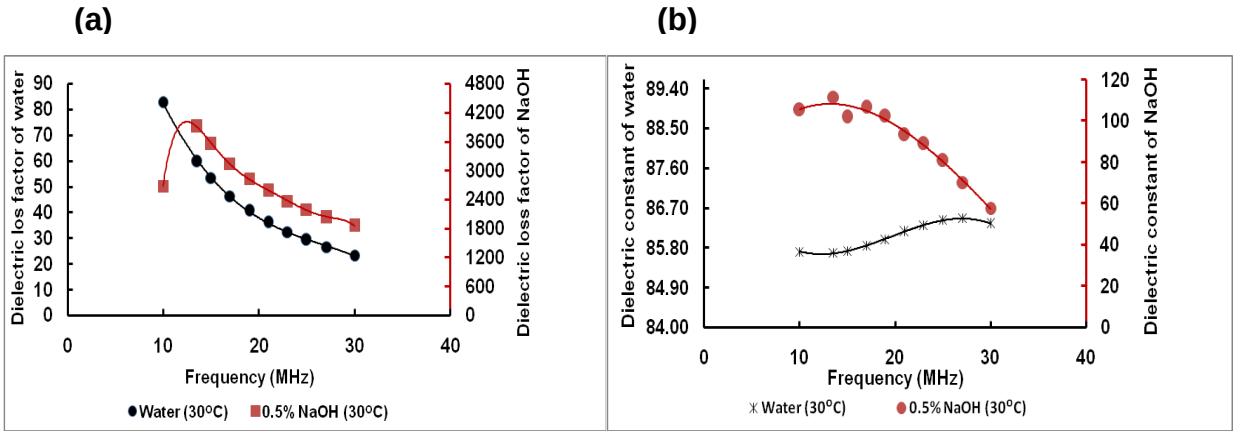


Figure A.2 Variation of the dielectric constant (a) and dielectric loss factor (b) of tap water and 0.5% NaOH solution at 30°C with frequency between 10-30 MHz.

The dielectric constant of tap water at 30°C increased with increasing frequency until it reached a maximum and decreased at a critical frequency f_c which is related to the relaxation time τ, where $f_c = 2\pi\tau$ whereas the dielectric loss factor decreased with increasing frequency. The dielectric constant of 0.5% NaOH solution at 30°C decreased with increasing frequency. While the dielectric loss factor increased until it reaches a maximum and decreased at a critical frequency *fc*. The dielectric constant and loss factor of the alkaline solution are higher than that of water because of the dissolved ions and solutes in the alkaline solution, which increased the ionic conductivity/dispersion and played a significant role in the RF dielectric heating. The differences in the dielectric properties might also be attributed to the differences in polarity. Water has higher polarity while NaOH solution has higher ionic conductivity. At lower frequencies (<200 MHz) such as the range of frequency used in this study, ionic conductivity played a dominant role, both ionic conductivity and dipole rotation of free water are important at higher frequencies such as MW frequencies (Tang et al. 2003; Wang et al. 2003). These researchers reported that polar dispersion controls the frequency characteristics of dielectric properties of pure liquids with polar molecules, such as water. A similar result was reported by Guan et al. (2004) involving the study of dielectric properties of mashed potatoes over 1 to 1800 MHz and 20°C to 120°C. Schubert and Regier (2005) also reported that at RF frequencies used in the industrial heating (10-50 MHz), the dielectric loss factor is mainly the result of ionic dispersion, which can be estimated directly from the measurement of electric conductivity (such as capacitance and

resistance), while, polar dispersion (resulting from water molecules) is responsible for the loss factor at higher frequencies (such as MW frequencies: 400-3000 MHz).

Figure A.3 illustrates the response of the biomass-alkaline mixture with particle size of 0.8 mm using ratio of 1:9 at 90^{o}C, with 30 minutes soaking time, and 1% NaOH solution. Both $\varepsilon_r^{'}$ and $\varepsilon_r^{''}$ decreased with increasing frequency. This shows that the interraction between biomass and NaOH solutionn produces different dielectric behavoir unlike the case where only NaOH solution is subjected to electromagnetic field. NaOH concentration plays a significant role (p=0.01) in the dielectric properties of lignocellulosic biomass straw. The dielectric constant and loss factor are higher at lower frequencies because at low frequency the polar molecules from water and the dissolved ions and solutes from the alkaline solution have enough time to align with the electric field generated by the radio frequency pulse. A similar finding has been reported by Izadifar et al. (2008). Gabriel et al. (1998) reported that pure alcohols (such as methanol and ethanol) attained maximum dielectric constant at low frequencies as a result of proper alignment of the alcohol molecules with the electric field. Dielectric property is a function of frequency, primarily because polarization is affected by frequency. A low dielectric constant has a low permittivity, or low ability to polarize and hold charge. A high dielectric constant, on the other hand, has a high permittivity, because high dielectrics are good at holding charges. However, precaution should be observed when using lower frequency during the RF pretreatment process so as to avoid thermal run away and thermal local degradation of lignocellulosic biomass straw, since at lower frequency, there is high acceleration of heating process of the material. The power penetration depth decreased with increasing frequency. A similar result was reported by Guan et al. (2004) with smashed potatoes at five levels of frequency (27, 40, 433, 915, and 1800 MHz) and moisture content of 81.6 to 87.8% w.b. Power penetration depth gives a good indication in selection of the right sample thickness during the RF and MW heating processes.

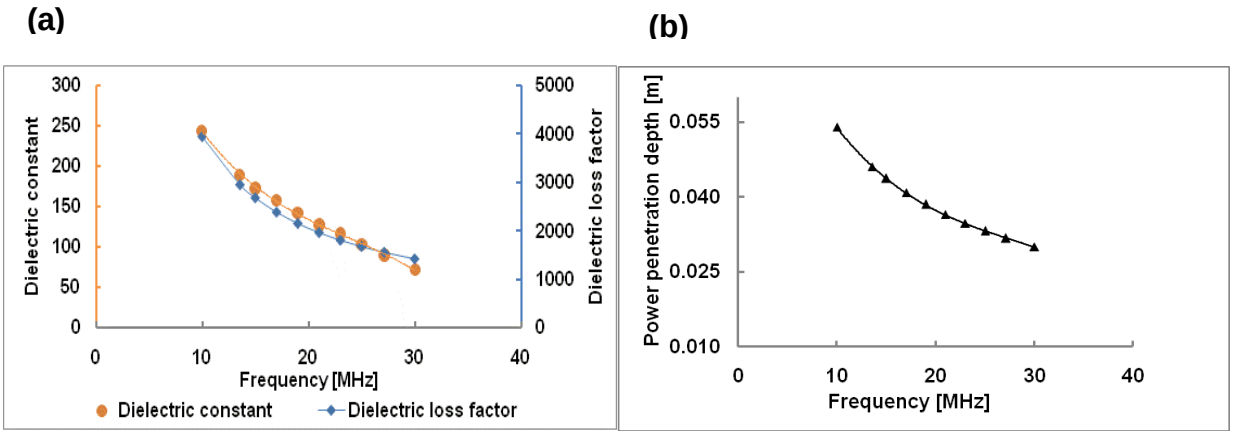


Figure A.3 Effects of frequency on the dielectric constant and loss factor (a) and power penetration depth on biomass-alkaline mixture with particle size of 0.8 mm, ratio of 1:9, at 90^{o}C, and 1%NaOH solution.

A.4.3 Temperature Effects

The effects of temperature on the dielectric properties are dependent on a number of factors, which comprises the frequency and composition. Figure A.4 illustrates the variation of the dielectric properties of the biomass-alkaline mixture with temperature at three levels of NaOH concentration, at 27.12 MHz using ratio 1:9 with 30 minutes soaking time. For the three levels of alkaline concentration (0, 0.5, and 1%), the dielectric constant decreased with increasing temperature (Figure A.4a). Increasing the temperature resulted in increased Brownian movement with decreased in fluid viscosity, which in turn reduced the static dielectric constant, and the relaxation time. This implies that for any given electromagnetic field intensity, higher temperature samples will absorb less energy as compared to the cooler ones; this also results in improved heating uniformity (Von Hippel 1954; Schubert and Regier 2005). Whereas, the dielectric loss factor increased with temperature over the three levels of alkaline concentration (Figure 4.Ab). However, the dielectric loss factor using 1% NaOH solution increased until it reached the maximum and decreased at the critical temperature T_c. At low frequencies, increasing the temperature increased the dielectric loss factor because of the noticeable role of ionic conduction at low frequencies. This may also be attributed to the higher solubility of solutes such as the NaOH into the biomass mixture at higher temperature. This observation is in agreement with the trends reported by Wang et al. (2003) on fruits and insects pests and also

Guan et al. (2004) on smashed potatoes. However, at high frequency band, increasing temperature reduced the dielectric loss factor. The electric conductivity in ionic solutions increased with temperature due to the decreased in the viscosity and increased in the mobility of the ions. This enhanced the conductivity of the ionic compounds and subsequently increased the dielectric loss factor (Stogryn 1971). This explains while the dielectric properties of ice and frozen material/foods are usually very low because water molecules in ice are immobilized within the matrix and behaves like bound water unlike free water (Tang et al. 2005). Figure A.4c demonstrates the inverse relationship of power penetration depth to the process temperature. Power penetration depth decreased as the temperature increased; except for the case of 0% NaOH that tend to increased at 90^{o}C. Ryynanen (1995) reported that power penetration depth becomes increasingly shallow at higher temperature. This study showed that 0% NaOH has higher power penetration than 0.5% and 1% NaOH concentrations. This implies that the dipole molecules from water have higher power penetration depth than the ionic dispersion from NaOH solution. At frequency of 27.12 MHz and ratio of 1:9, this study depict that the interaction between concentration and temperature has a significant effect ($p=0.01$) on the dielectric properties of lignocellulosic biomass.

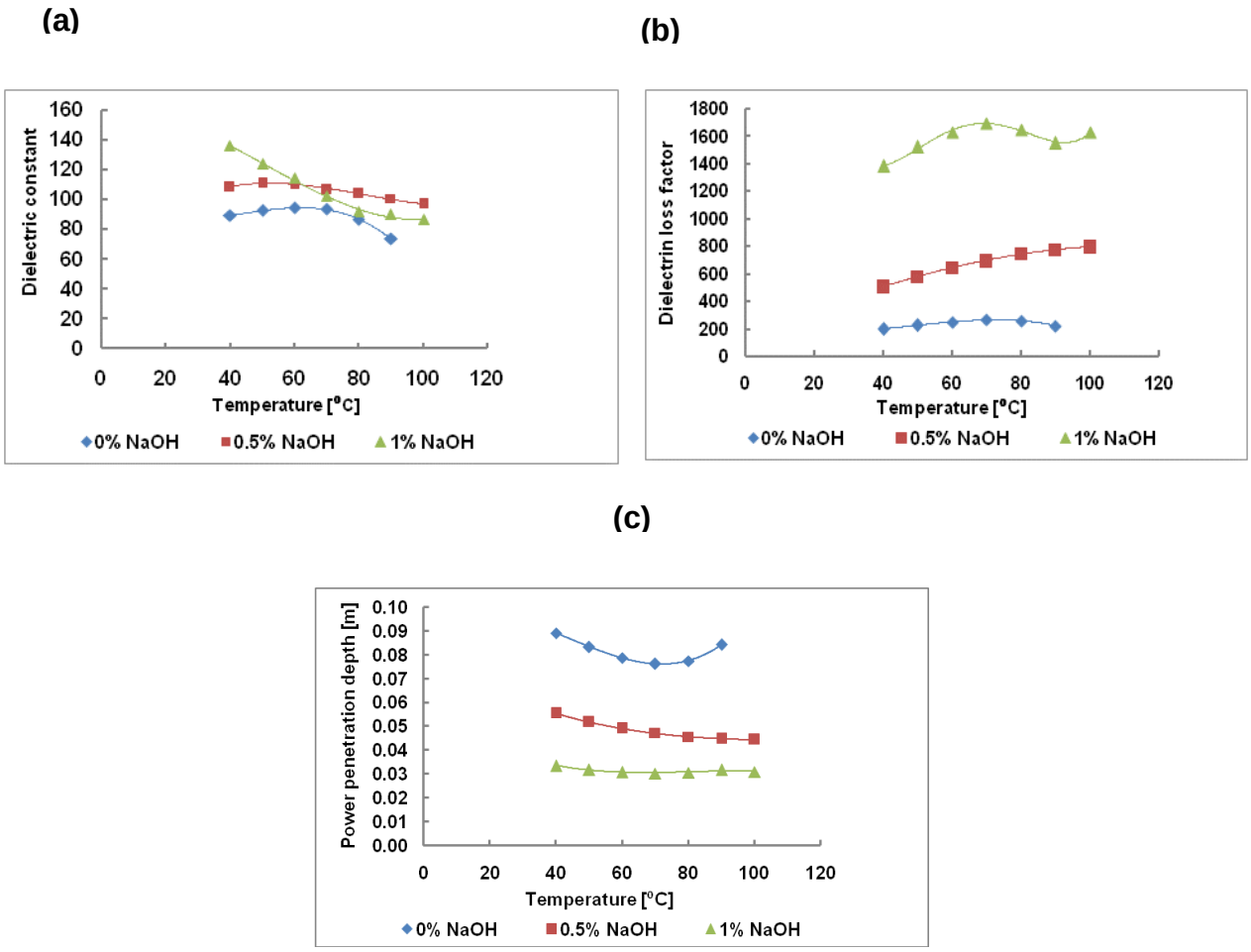


Figure A.4 Variation of the dielectric properties of the biomass-alkaline mixture with temperature at three levels of NaOH concentration, 27.12 MHz using ratio 1:9 with 30 minutes soaking time

A.4.4 Moisture Content and NaOH Concentration Effects

Figure A.5 shows the variation of dielectric properties of biomass straw with five levels of moisture content (85, 87, 89, 90, and 91% w.b.), using particle size of 0.8 mm, frequency of 27.12 MHz, with 30 minutes soaking time, and at 90°C and 50°C. This represents the 0% NaOH concentration, where tap water was used as a solvent and added to the biomass. The moisture content of biological materials has a great influence on the dielectric properties. The polar molecules and the ionic compounds played significant roles in polarization and dissipation of the electromagnetic energy in the medium. Figures A.5a and A.5b depict that the dielectric constant

and loss factor at 50^{o}C and 90^{o}C, respectively. Both parameters increased with moisture content until it reached the maximum where the effect of additional moisture content diminished. At low levels of biomass moisture content, the dielectric constant and loss factor are low. This could be attributed to the low mobility of the ions, solute, and water molecules which are bound to the biomass particles. As the moisture content increased, the mobility of the molecules increased and resulted to a corresponding increased in both parameters at 50°C. As the moisture content increased, the water in the biomass straw act as free water in intercellular spaces of the biomass straw, with more capacity to convert the electromagnetic energy into thermal energy. Free water has higher dielectric properties than the bound water (Feng et al. 2002; Schubert and Regier 2005). These authors reported that between 22 and 60°C, the dielectric properties of foods extremely decreased with decreasing moisture content. Izadifar and Baik (2009) also reported that the dielectric loss factor of rhizome packed bed increased with increasing particle moisture content at temperatures range of 25 to 54^{o}C using 27.12 MHz radio frequency. However, in this study, at higher temperature (90°C), the dielectric constant and dielectric loss factor increased with moisture content until it reaches maximum and decreased at the critical moisture content M_c. This may be attributed to the chemical composition such as the polysaccharides (cellulose and hemicellulose) and the phenolic compound (lignin) present in the lignocellulosic biomass straw. This may also be associated with the evaporation or lost of the water molecules at higher temperature. Excessive increase in moisture content may also result to washing down most of the dissolved ions and solutes to the bottom of the biomass bed, thereby leading to lower dielectric properties. This study showed that the critical moisture content is dependent on the process temperature. At higher temperature (90°C) less moisture content, 87% (w.b.) was required to attain high dielectric properties, unlike 90% (w.b.) that was needed at 50°C. Figure A.5c shows the inverse relationship of power penetration depth to the five levels of moisture content. The penetration depth decreased as the moisture content increased and decreased as the temperature increased. Ryynanen (1995) reported that power penetration depth becomes increasingly large at lower moisture content.

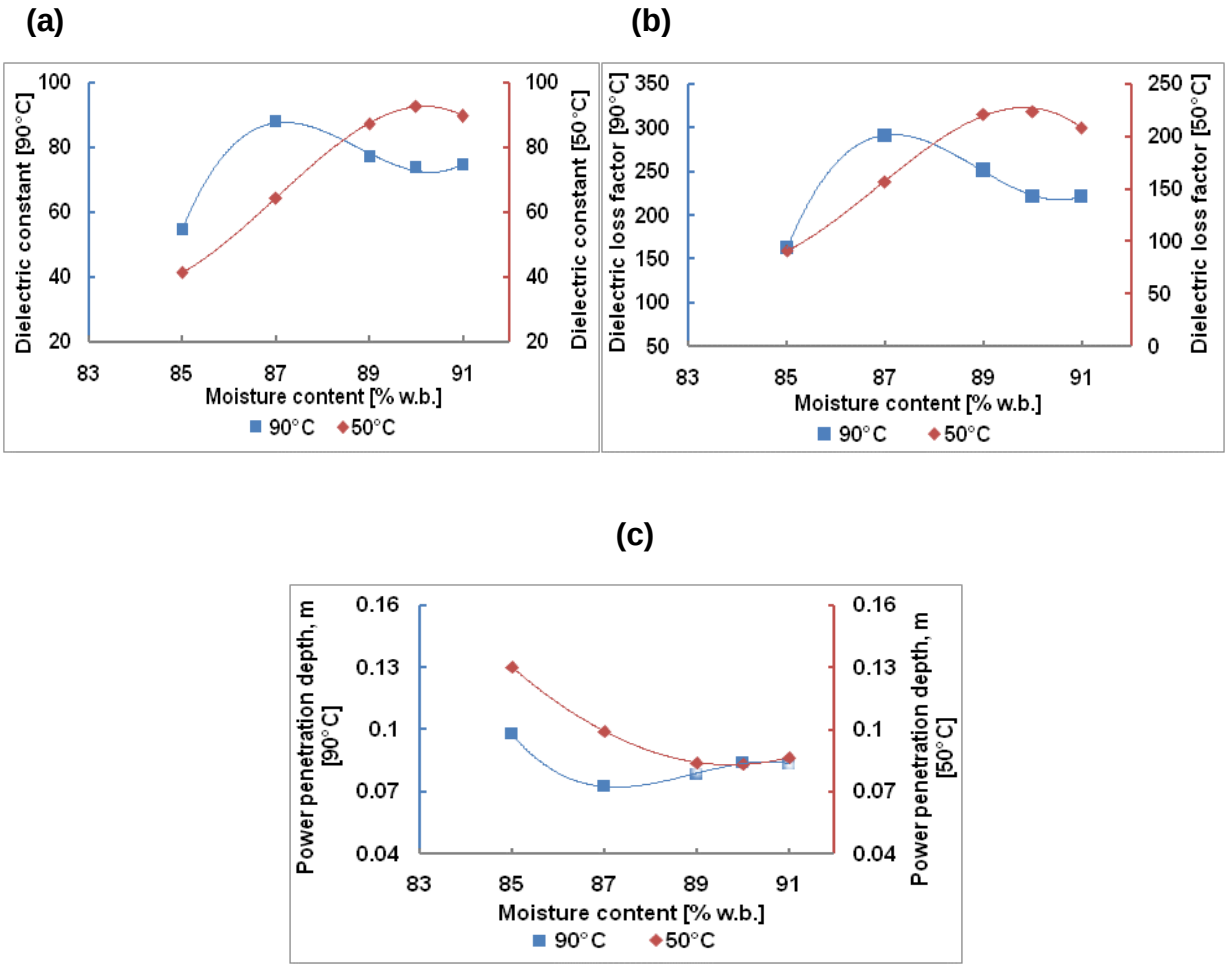


Figure A.5 Variation of dielectric properties of biomass straw with five levels of moisture content (85, 87, 89, 90, and 91% w.b.), using particle size of 0.8 mm, frequency of 27.12 MHz, with 30 minutes soaking time, and at 90°C and 50°C.

Figure A.6 shows the variation of dielectric properties of biomass straw with three levels of NaOH concentration (0, 0.5, & 1%), using particle size of 0.8 mm, frequency of 27.12 MHz, ratio 1:9 of biomass-alkaline mixture, and at 50°C. This study revealed that the dielectric properties are dependent on the NaOH concentration. The dielectric constant and loss factor increased as the NaOH concentration increased. This could be attributed to the movement of the ionic compounds from the NaOH solution, and also the type and quantity of molecules and ions transferred or released from the solid to the liquid phase, and subsequently occupies the pores of the biomass bed. Furthermore, the interaction between the polar and ionic compounds in the biomass bed and depending on the solubility of the solutes in the mixture could result to a

significant response to the electrical field created during the RF heating. Ryynanen (1995) reported that solutes and ions in the liquid phase have higher mobility and subsequently resulted to higher dielectric loss factor. This interaction is different depending on the NaOH concentration and the temperature. Ryynanen (1995) reported that dielectric loss factor is strongly dependent on the ionic loss component, while ionic conductivity is highly dependent on temperature. In this study, higher NaOH concentration resulted to higher dielectric constant and loss factor due to the presence of more solutes and ions. Figure A.6c illustrates the inverse relationship of power penetration depth with NaOH concentration-biomass at 50°C. Increase in the NaOH concentration resulted in decreased in the power penetration depth. From Figure A.6a, 1% NaOH concentration produced the highest dielectric loss factor. This implies that the higher the dielectric loss factor, ε_r'' the less deep the electromagnetic energy will penetrate into the material. A similar result has been reported by Buffer (1993). Guan et al. (2004) demonstrated that increasing the salt concentration of smashed potatoes reduced the power penetration depth. Power penetration depth describes how far into a material radio frequency energy penetrates before it decreased to a certain fraction of its original value. This parameter is important because it represents guiding principle to the heating effectiveness of the material.

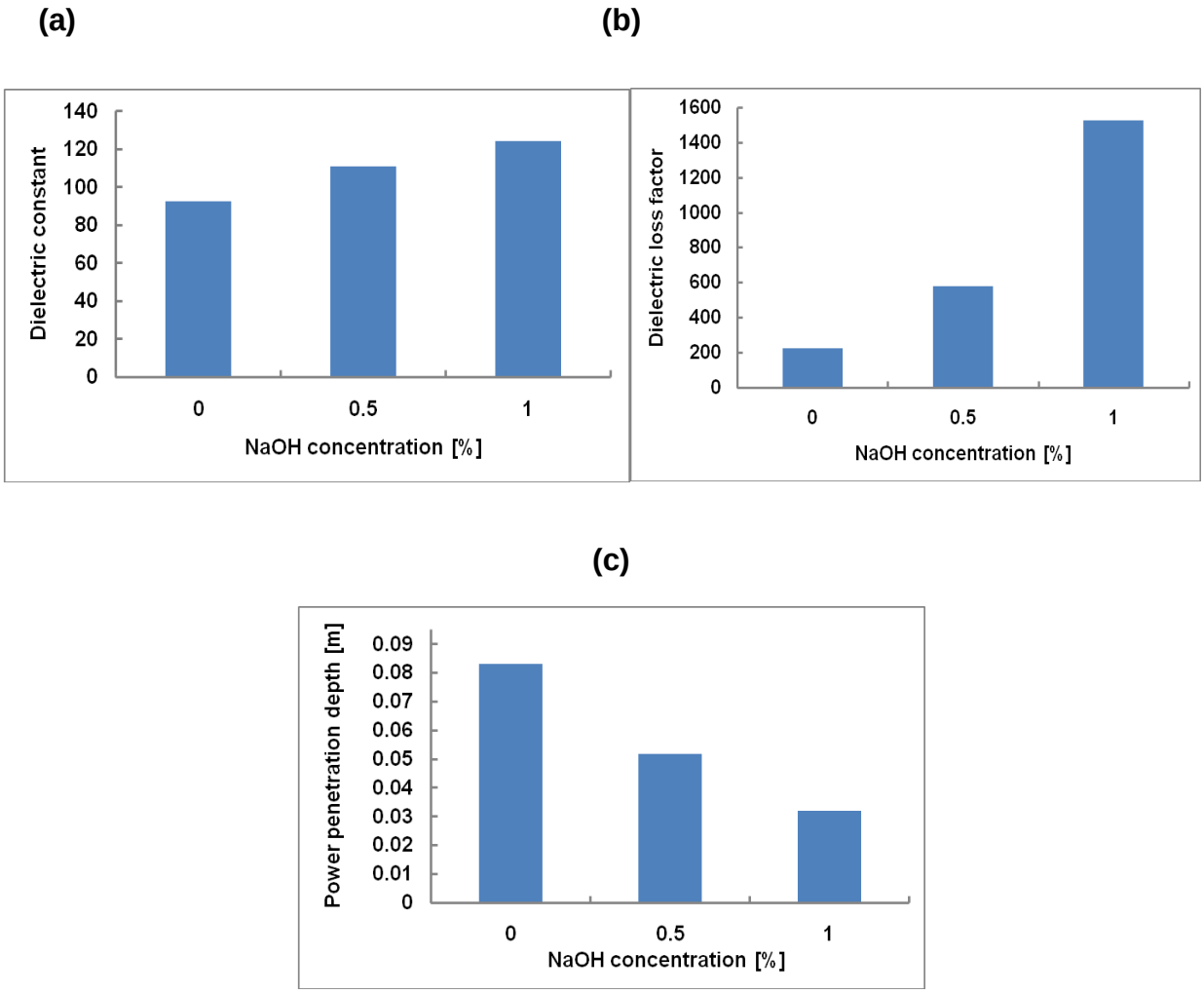


Figure A.6 Variation of dielectric properties of biomass straw with three levels of NaOH concentration (0, 0.5, & 1%), using particle size of 0.8 mm, frequency of 27.12 MHz, ratio 1:9 of biomass-alkaline mixture, and at 50°C.

A.4.5 Particle Size Effects

To further understand the dielectric behavior of lignocellulosic biomass matrix, two different particle sizes 0.8 and 1.6 mm were used at 27.12 MHz with biomass to NaOH solution ratio of 1:7 using 1% NaOH concentration at 90^{o}C with 30 minutes soaking time. Figure A.7 shows the variation of dielectric properties of lignocellulosic biomass-NaOH mixture with two levels of particle size. Particle size was found to have a substantial influence on the dielectric properties of lignocellulosic biomass with significant effect of p=0.01. The larger particle size (1.6 mm)

proved to produce higher dielectric constant and dielectric loss factor (Figure A.7a & A.7b). This may be attributed to the absorption level of the dissolved ions and solutes from the liquid phase by the larger particle size as compared to the smaller particle size. The larger particle size has more porosity than the smaller particle size, as such more dissolved ionic compounds and solutes will fill up such pores. Higher porosity created by the larger particle size improves the mobility of the dissolved ions and solutes within the pores of the bed and can be polarized easily and better in the RF electromagnetic field. This subsequently enhances the dielectric constant and dielectric loss factor. Izadifar and Baik (2009) reported that the dielectric constant of packed bed of rhizome particles with a porosity of 70.13% seemed to be higher than packed bed with a porosity of 60.40%. Based on the observation from this study, it showed that the effect of porosity is strongly dependent on the chemical and material composition. It is interesting to know that 1.6 mm particle size generated more dielectric constant and dielectric loss factor than the 0.8 mm particle size. This might also be attributed to the presence of free solution that exist in the bed of larger particles (1.6 mm) unlike the bound solution that exist in the smaller particles (0.8 mm). This information is important in the RF heating pretreatment process because it will help in saving the cost of particle size reduction, which is one of the energy intensive stages involved in the initial preparation of the RF pretreatment technique. Figure A.7c illustrates the relationship of power penetration depth with particle size. The smaller particle size proved to have more penetration depth than the larger particle size. This might be due to better heat transfer rate associated with smaller particle size, which subsequently enhanced the penetration depth of the biomass bed as compare to the higher particle size. As the particle size increased the average heat-transfer rate diminished. This may be explained by the fact that with different particle sizes the heat conduction of the medium of the biomass bed changed as a response to the physical and structural changes of the particles.

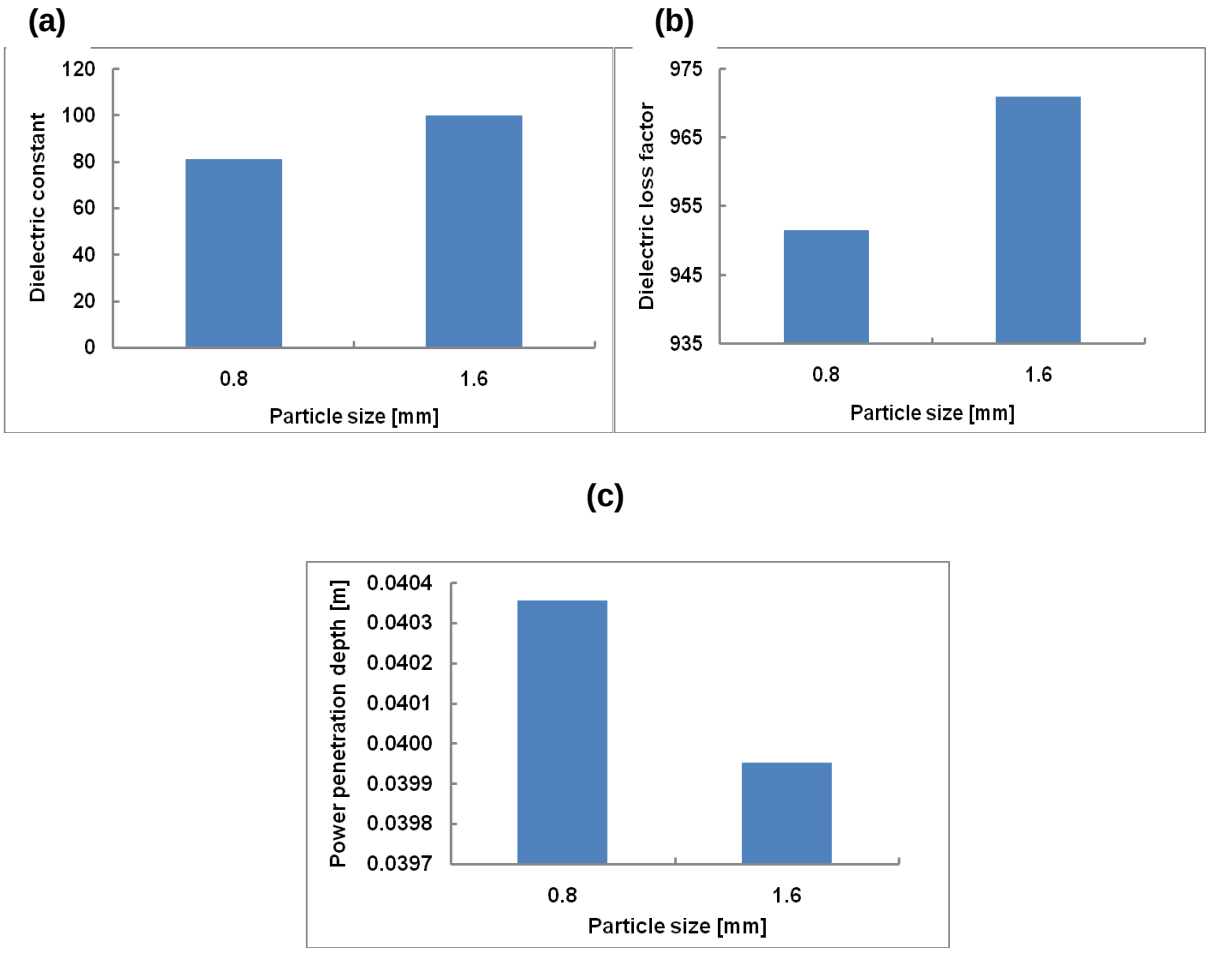


Figure A.7 Variation of dielectric properties of lignocellulosic biomass-NaOH mixture with two levels of particle size, 27.12 MHz, ratio of 1:7 using 1% NaOH concentration at two levels of temperature (90^{o}C) with 30 minutes soaking time.

A.4.6 Soaking Time Effects

The response of dielectric properties with soaking time of the biomass-NaOH solution mixture was investigated. Figure A.8 illustrates the changes of dielectric properties using ratio 1:9 of biomass to 1% NaOH solution mixture at 90^{O}C using 27.12 MHz, particle size of 0.8 mm, with two levels of soaking time (zero and 30 minutes). As observed in this study, as the soaking time increased from zero to 30 minutes, the solvent (NaOH solution) is highly absorbed by the biomass particle and more solutes and ions are transferred from the liquid phase to the solid phase and vice versa. Since the dissolved ions and solutes are transported into the particles and

pores of the biomass bed, there was significant increase in the dielectric constant and loss factor of the mixture. This study indicated that it is important to allow an extended length of time for the interaction between the complex polysaccharides such as cellulose, hemicellulose, and lignin in the biomass straw with NaOH solution and water. Soaking time has significant effect ($p=0.01$) on the dielectric behavior of the lignocellulosic biomass matrix when subjected to electromagnetic field. This study would be useful in designing and formulation of functional RF pretreatment processes and for developing predictive RF pretreatment models. Especially, for the optimization of delignification of biomass matrix, so as to increase the accessibility and digestibility of the energy potentials (cellulose and hemicellulose) in biomass straw. Figure A.8b illustrates the inverse relationship of power penetration depth with soaking time. The penetration depth decreased as the soaking time increased from zero to 30 minutes. Buffer (1993) also reported that the higher the dielectric loss factor, the less deep the electromagnetic energy will penetrate into the material.

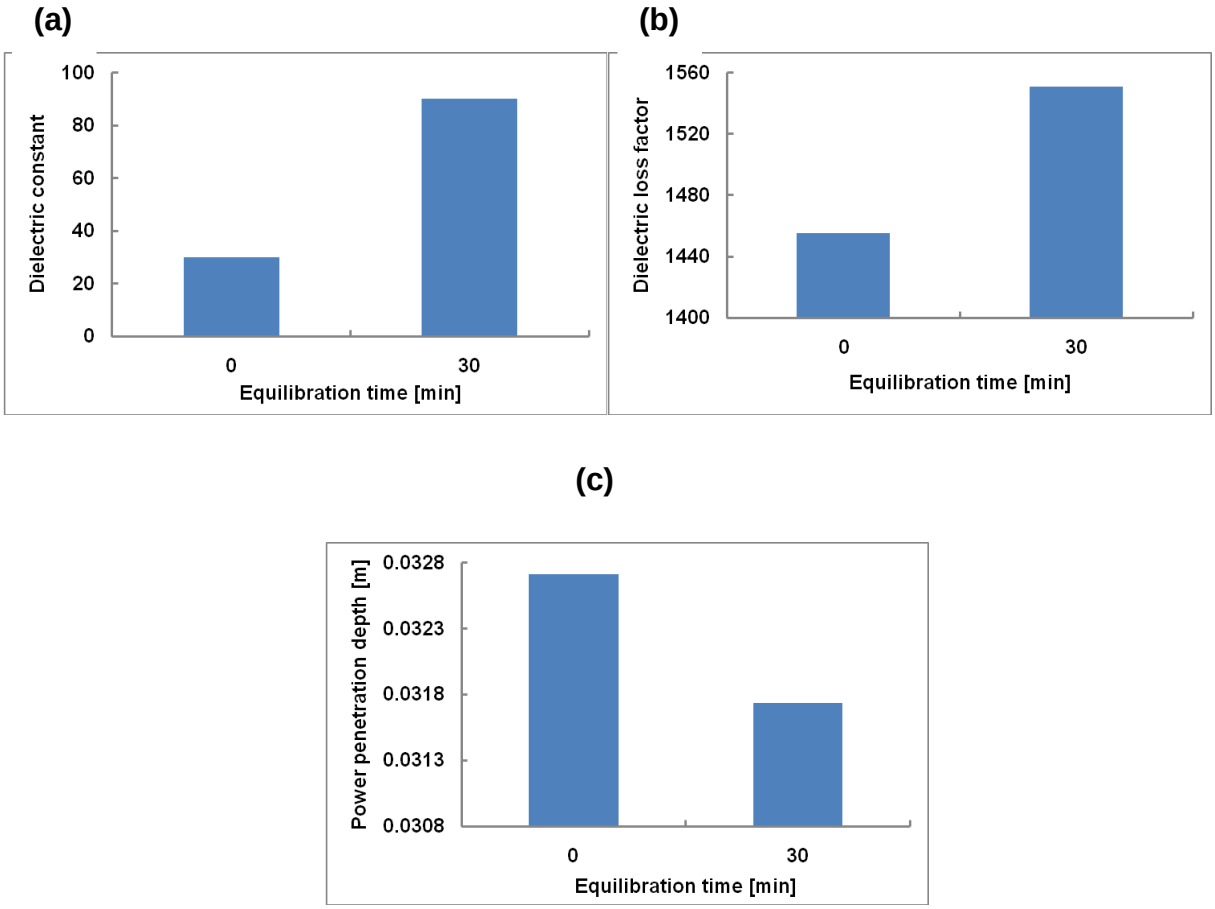


Figure A.8 Variation of dielectric properties using ratio 1:9 of biomass to 1% NaOH solution mixture, at 90^{O}C, 27.12 MHz, with particle size of 0.8 mm, and two levels of soaking time.

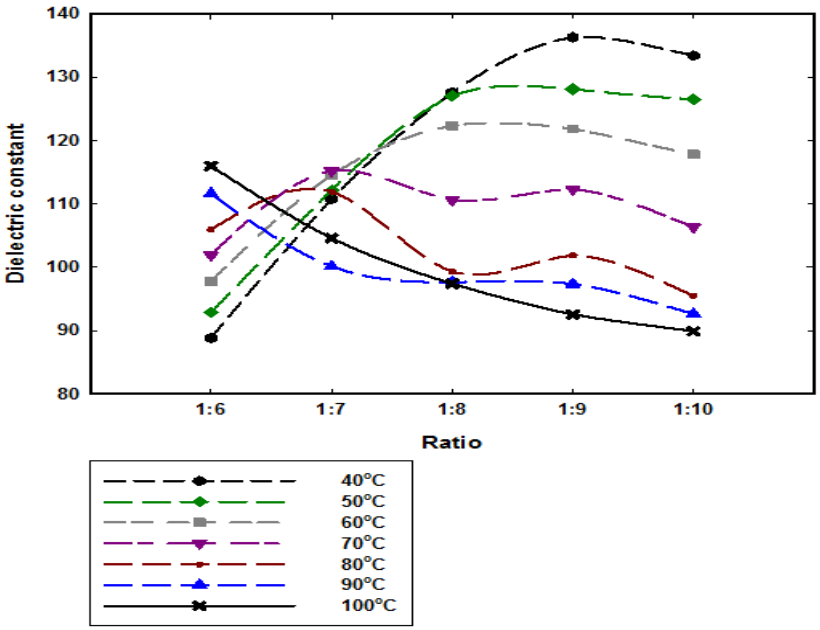
A.4.7 Ratio of Biomass to NaOH Solution Effects

To better understand the dielectric behavior of lignocellulosic biomass straw, it is also important to know the right amount of biomass that is required to be mixed with a given NaOH solution. Figure A.9 illustrates the variations of dielectric properties with five levels of ratio of biomass to 1% NaOH solution at seven levels of temperatures using 1.6 mm particle size based on 27.12 MHz frequency. The observation from this study showed that dielectric properties are strongly dependent on the ratio of biomass-NaOH solution mixture. It is also interesting to know that optimal ratios of the mixture are also associated with the operating temperature. The dielectric constant and dielectric loss factor increased with increasing ratio until it reached maximum and

decreased at the critical ratio R_c. At critical ratio, additional amount of NaOH solution into the biomass has no further and significant effect on the dielectric behavior, because at this critical ratio the system is so saturated with the required ions, solutes, and dipole molecules which are also dependent on the process temperature. Therefore, different ratios have different critical or optimal ratios depending on the operating temperature. This study also confirms that lower temperatures generate higher dielectric constant as compared to higher temperatures. The highest dielectric constant was obtained with ratio 1:9 at 40^{o}C. At the following temperatures 50^{o}C, 60^{o}C, 70^{o}C, 80^{o}C, 90^{o}C, and 100^{o}C the optimal ratios are 1:8, 1:8, 1:7, 1:7, 1:6, and 1:6, respectively (Figure A.9a). This indicates that different temperature requires different optimal ratios to produce optimal dielectric constant. This observation suggested that in RF pretreatment process, consideration need to be given to the ratio of biomass to alkaline solution depending on the operating temperature so as to realize higher dielectric constant. As the process temperature increased the ratio required to generate optimal dielectric constant decreased. This information might be helpful in decreasing the cost of chemicals use during the RF pretreatment, as well as the cost of generating unnecessary heating during the pretreatment, which might result to local heating and subsequently thermal degradation of sugars in the biomass particles. Kumar et al. (2009) reported that alkaline pretreatment utilizes lower temperatures and pressures. Sugar degradation must be avoided to prevent the formation of inhibitors such as furfural, which inhibits enzymatic activities during the fermentation process of bio-ethanol production. Figure A.9b also illustrates the dependency of dielectric loss factor on the ratio of biomass-NaOH solution with respect to the process temperature. It can be seen that the dielectric loss factor increased with increasing ratio and temperature until it reached maximum and decreased at the critical ratio and temperature (1:9 and 70^{o}C, respectively). The highest dielectric loss factor was obtained at 70^{o}C and at ratio of 1:10. Figure A.9c also illustrates the variation of power penetration depth with ratio and temperature. Penetration depth has inverse relationship with ratio and temperature. As the ratio and temperature increased, the penetration depth decreased. However at higher temperatures (80, 90, & 100^{o}C) the dielectric behavior changes rapidly and exhibits a random response. Form this study; it has been observed that the process condition that provides optimal dielectric constant does not guaranty optimal dielectric loss factor and optimal power penetration depth for the RF biomass pretreatment. Based on this trade off, this means that

a balance needs to be sourced for and a compromised required to be made between dielectric constant and dielectric loss factor. It would not be cost effective if all the stored electrical energy cannot be converted to thermal energy within the biomass for the delignification process. Therefore, the best strategy will be keeping as much thermal energy that was obtained from the converted electrical energy during the RF pretreatment process. Based on the results obtained from this study, 60^{o}C at ratio 1:8 seems to be the optimal process parameter that will generate optimal condition for the RF pretreatment process.

(a)



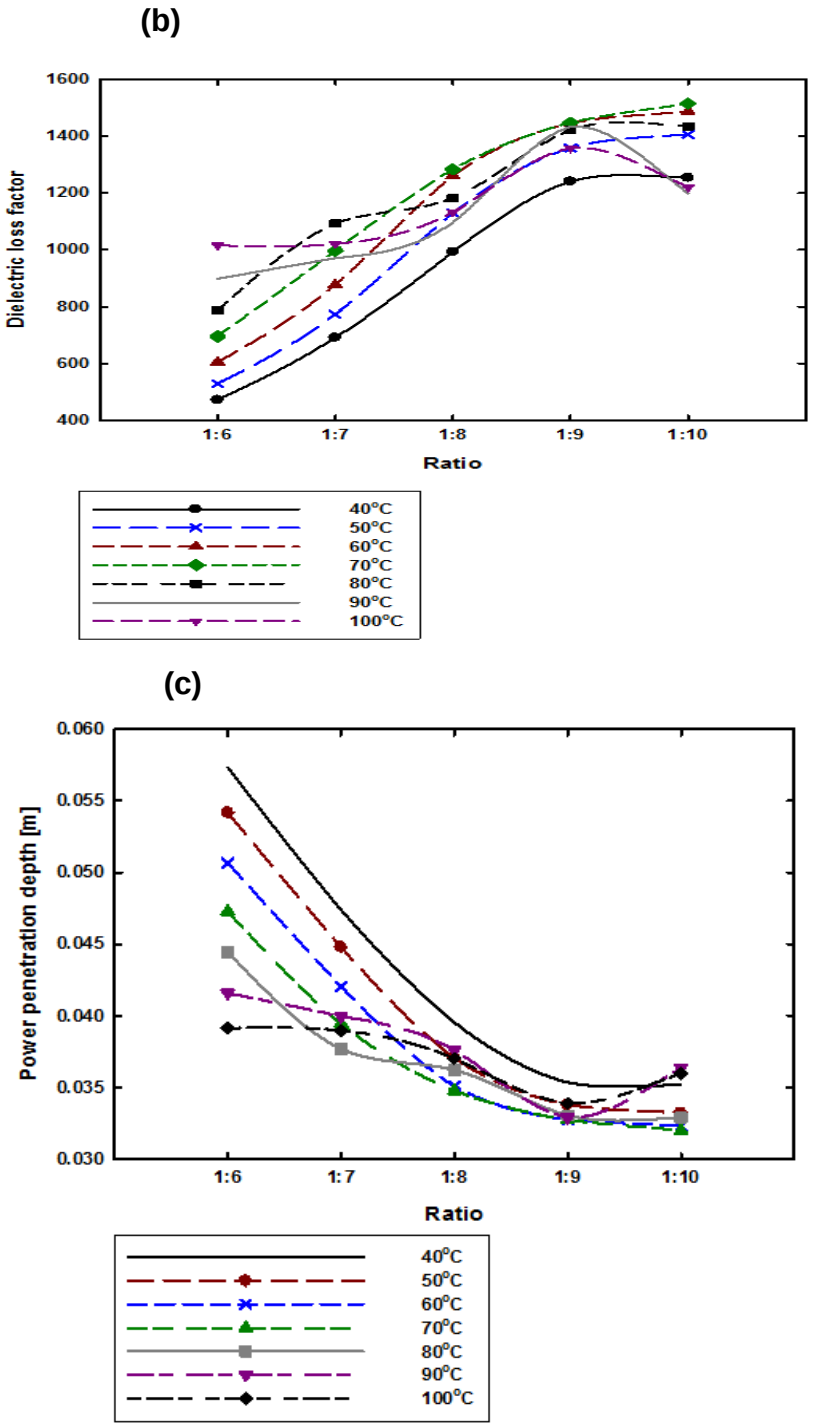


Figure A.9 Variations of dielectric properties with five levels of ratio of biomass to 1% NaOH solution at seven levels of temperatures using 1.6 mm particle size based on 27.12 MHz frequency

A.4.8 Statistical Analysis

Table A.8 depicts the regression equations with significant terms. The following parameters; NaOH concentration, particle moisture content, temperature, particle size, soaking time, and ratio of biomass to NaOH solution have significant effects on the dielectric constant, dielectric loss factor, and power penetration depth on lignocellulosic biomass barley straw.

Table A.8 Multiple regression equations for the dielectric constant and dielectric loss factor of biomass bed at 13.56 and 27.12 MHz

Frequency (MHz)	Regression equation	MSE[a]	DF[b] of Error	Adjusted R^2
Dielectric constant				
13.56	$\varepsilon_r' = -85.279 + 39.683R + 78.001C + 0.007T + 51.43P$	134.516	68	0.959
27.12	$\varepsilon_r' = 22.7 + 15.157R + 13.72C - 0.165T + 19.468P$	34.823	68	0.929
27.12	$\varepsilon_r' = 135.237 - 0.854T + 0.484E + 16.395P$	19.237	6	0.956
Dielectric loss factor				
13.56	$\varepsilon_r'' = -2476.627 + 635.108R + 1739.786C + 8.669T - 2.704P$	8.95×10^{-6}	68	0.993
27.12	$\varepsilon_r'' = -1320.039 + 338.165R + 918.648C + 4.538T + 4.066P$	4496.522	68	0.982
27.12	$\varepsilon_r'' = 1415.7 + 2.39T + 7.257E - 264.37P$	2576.217	6	0.916

[a] mean square error
[b] Degree of freedom
R=ratio, C=concentration, T=temperature, P=particle size, E=soaking time.

A.5 Conclusion

Dielectric properties are strongly influenced by biomass particle moisture, frequency, temperature, NaOH concentration, biomass particle size, soaking time, and ratio of biomass to alkaline/water solution. Dielectric properties of lignocellulosic biomass-alkaline mixture decreased with increasing frequency. The dielectric constant decreased with increasing temperature, while the dielectric loss factor increased with increasing temperature. However, the dielectric loss factor using 1% NaOH solution increased until it reached the maximum and decreased at the critical temperature, T_c. Dielectric constant and dielectric loss factor at 50°C increased with increasing particle moisture content until it reached a maximum where the effect of additional moisture content diminished. Observation from this study shows that high dielectric properties can be obtained at low temperature depending on the other process parameters. Dielectric constant and dielectric loss factor increased with increasing NaOH concentration, particle size, and soaking time. The dielectric constant and dielectric loss factor increased with increasing ratio until it reached maximum and decreased at the critical ratio R_c. The critical ratio depends on the operating temperature. For all cases, the power penetration depth had an inverse relationship with all the tested material and process variables. Penetration depth decreased as any of the material or process variable increased. This study would be useful in designing and formulation of functional RF pretreatment processes and for developing predictive RF pretreatment models. Especially, for the optimization of delignification process of biomass matrix, so as to increase the accessibility and digestibility of the energy potentials (cellulose and hemicellulose) in biomass straw during the enzymatic hydrolysis and fermentation process.

Appendix B

B. Thermal Properties of Lignocellulosic Biomass

Barley Straw

A similar version of this Appendix has been presented and published in an ASABE international conference:

- Iroba, K.L., L.G. Tabil, V. Meda and O.D. Baik. 2013. Thermal properties of lignocellulosic biomass barley straw. ASABE Annual International Meeting, Kansas City, MO. Paper Number: 1594972, July 21-24.

B.1 Abstract

Lignocellulosic biomass barley straw is a complex formation of cellulose, hemicellulose, and lignin. The lignin acts as an external crosslink binding hemicellulose and cellulose with cellulose positioned at the inner core of the structure. The structure and composition of lignocellulosic makes it naturally resistant to enzymatic and microbial degradation during hydrolysis and fermentation processes. This necessitates pretreatment of lignocellulosic biomass prior to hydrolysis and fermentation. Pretreatment of lignocellulosic biomass involves the application of heat and the response to heat transfer is dependent on thermal properties of the biomass. Therefore, the essential thermal properties of lignocellulosic biomass required for optimization processes were determined. Thermal conductivity was measured using the line heat source method at three levels of temperature (4^{o}C, 25^{o}C, and 90^{o}C), and calculated using the maximum slope method. The specific heat capacity was investigated using the differential scanning calorimetry (DSC) at three levels of temperature (70^{o}C, 80^{o}C, and 90^{o}C). Both measurements was done with five levels of ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8) and

three levels of hammer mill screen size (0.354, 0.8, and 1.6 mm). Thermal diffusivity was calculated using thermal conductivity, specific heat, and bulk density values. The results obtained shows that temperature played a significant role in the thermal conductivity of biomass. Specific heat had a linear relationship with temperature at higher ratio of biomass:NaOH solution. Thermal diffusivity did not show any specific trend with the variables studied.

B.2 Introduction

Biofuel from lignocellulosic biomass (second generation) is one of the few options to complement fossil fuels in the long term, with full potential for greenhouse gas savings and energy security. The lignocellulosic nature of biomass requires initial pretreatment of the feedstock to enhance easy accessibility and digestibility of the energy potentials (cellulose and hemicellulose) present in the biomass. There are a number of pretreatment routes that could be used for the production of biofuel from biomass (Iroba and Tabil 2013a). Most common processes in biomass processing plant involve heating and cooling of the feedstocks. Thermal aided pretreatment (heat transfer), drying (heat and mass transfer), and evaporation (heat and mass transfer) are some of the unit operations found in biofuel processing. These unit operations involve the transfer of heat between a product and some heating or cooling medium (Singh and Heldman 2009). The response of biological materials to heat and mass transfer is dependent on their thermal properties.

Thermal properties of biomass such as thermal conductivity, specific heat, and thermal diffusivity are necessary to determine the optimal processing conditions of lignocellulosic biomass. They are invaluable considerations required in the design and modeling of biomass processing operations involving heat transfer. Drouzas and Saravacos (1988) and Emami et al. (2007) reported that thermal conductivity is affected by sample moisture, temperature, and bulk density.

The specific heat of biological materials has usually been investigated using ice calorimetry or the method of mixture (Dutta et al. 1988). These methods have the disadvantage of long time to stabilize, temperature control, and used only for a set of sample grains in the same bulk density.

Due to the limitations listed above, the differential scanning calorimeter (DSC), which is an accurate and rapid method has been used in recent time for determination of specific heat. Emami et al. (2007) used DSC to determine the specific heat of chickpea flour, isolated starch, and isolated protein as a function of temperature and moisture content. Izadifar and Baik (2007) have also utilized DSC in measuring the specific heat of the rhizome of *podophyllum peltatum* for drying and ethanol extraction.

Thermal conductivity of biological materials is determined in different ways. The line heat source method is the common and more accurate approach for the measurement of thermal conductivity of biological materials (Yang et al. 2002; Dutta et al. 1988). This method is based on transient heat transfer and uses a bare wire or thermal conductivity probe as heating source. The thermal conductivity probe is robust, easily calibrated, and can be used over a wide range of temperatures for many materials (Lobo and Cohen 1990). Thermal conductivity is calculated based on the rate of temperature rise within the bulk of the sample (Yang et al. 2002). Tabil et al. (2003) used this method for the determination of thermal conductivity of sugarbeet roots as a function of temperature. The probe method has also been applied on different areas, such as on small food samples (Sweat and Haugh 1974), liquids (Asher et al. 1989), and apples (Ramaswamy and Tung 1981).

Thermal diffusivity (α) can be measured using different approaches. The most widely used is the indirect method, which involves calculation using the experimental values of specific heat (C_p), thermal conductivity (k), and bulk density (ρ_b) from the following equation (Singh and Heldman 2009; Yang et al. 2002):

$$\alpha = \frac{k}{\rho_b C_p} \tag{B.1}$$

A perusal of pertinent resources revealed that there is limited information available on the thermal properties of lignocellulosic biomass barley straw grind. The objective of this study is to determine the thermal conductivity, specific heat, and thermal diffusivity of lignocellulosic biomass barley straw grind as a function of temperature, ratio of biomass:NaOH solution (liquid

content), and hammer mill screen size (bulk density). Thermal properties information is required in modeling heat transfer during RF-alkaline pretreatment of lignocellulosic barley biomass.

B.3 Materials and Methods

B.3.1 Sample Procurement and Preparation

Barley straw was obtained in October, 2009 from RAW Ag Ventures Limited, Maymont, SK. The straw was ground using a hammer mill (Serial no. GM13688; Glen Mills Inc., Maywood, NJ) and precision grinder (Falling Number, Model No. 111739, S-141 05 Huddinge, Sweden) with screen sizes of 0.8, 1.6, and 0.354 mm to increase the surface area of the biomass. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation and to provide flow of the biomass in and out of the hammer mill. The initial moisture content of the straw was 8.8% (d.b.). The moisture content was measured based on ASABE standard method S358.2 DEC1988 (2008). Based on the required ratio, the right amount of biomass grind and NaOH solution were measured and mixed together for the thermal properties measurement. For details of sample preparation, see Iroba et al. (2013).

B.3.2 Particle and Bulk Density Measurement

The particle density of the samples was measured using the gas multi-pycnometer (model No. MVP-2; QuantaChrome, Boynton Beach, FL) by calculating the displaced volume of nitrogen gas by a known mass of material. Three replicate tests were performed on each sample. The bulk density of the samples was determined by passing the material through a funnel which was placed above a standard 0.5 liter steel cup (SWA 951, Superior Scale Co. Ltd., Winnipeg, MB). Blockages in the funnel during ground biomass flow were cleared using a thin steel rod. After filling the cup, the excess sample was removed by passing a steel roller in a zigzag manner over the top of the cup. The mass contained in the cup was then determined. Bulk density was calculated by dividing the mass of material within the cup by the volume of the cup. Three replicates were performed for each sample. The porosity of the biomass was also calculated, for details of the calculation of the physical characteristics; see Iroba et al. (2013).

B.3.3 Thermal Conductivity Measurement

Thermal conductivity of lignocellulosic biomass was measured based on the line source method (Lobo and Cohen 1990). This approach is suitable and preferred to other methods such as the steady state method, because the later takes very long time (4-24 h) to obtain a reliable data (Lobo and Cohen 1990). The characteristics of lignocellulosic biomass frequently change as a result of degradation when exposed to high temperatures over a long period of time needed for the measurements. The line source method was designed based on the consideration of a cylinder of "infinite length, with an infinitesimally thin, infinitely long heating element located along its axis" (Lobo and Cohen, 1990). A constant finite quantity of heat $Q^{'}$ is generated per unit length of the heating element within a short specified period of time. The linear relationship between temperature change (T_2-T_1) and natural logarithm of heating time ln(t) is given by Lobo and Cohen (1990) as shown in equation B.2:

$$T_2\text{-}T_1 = \frac{Q^{'}}{4\pi k}\ln\left(\frac{t_2}{t_1}\right) \quad \text{(B.2)}$$

k = thermal conductivity of the sample (W m^{-1}°C^{-1})

$Q^{'} = I^2R$ = heat input per meter of line source (W m^{-1}),

T = temperature (°C), and

t = time (s)

subscripts 1 and 2 are the initial and final values

B.3.3.1 Probe Construction

A thermal conductivity probe was constructed from a cylindrical brass tube with 1.6 mm outer diameter and 96 mm in length. Figure B.1 shows the schematic diagram of the probe used in this study. A single insulated constantan heating wire with a diameter of 0.254 mm was passed through the length of the tube heating element. Constantan is used as the heating element due to its low temperature coefficient of resistivity (Lobo and Cohen 1990). The end of the tube was sealed with a silver solder which can resist temperatures as high as 210°C. Lobo and Cohen (1990) reported that J-type thermocouple is needed when a certain amount of sensitivity is

necessary in the temperature measurement (with small temperature changes). A J-type thermocouple (iron-constantan, 0.127 mm diameter) was inserted halfway into the tube. The remaining space in the tube was filled and the wires were all coated with a high thermal conductivity paste (Wakefield Engineering Inc., Wakefield, MA). Heat-shrink wrappers, polytetrafluoroethylene (PTFE) thread seal tape and a ceramic fiber rope were used to connect the tube to the extended wires to keep the heating wire and thermocouple stable and in place.

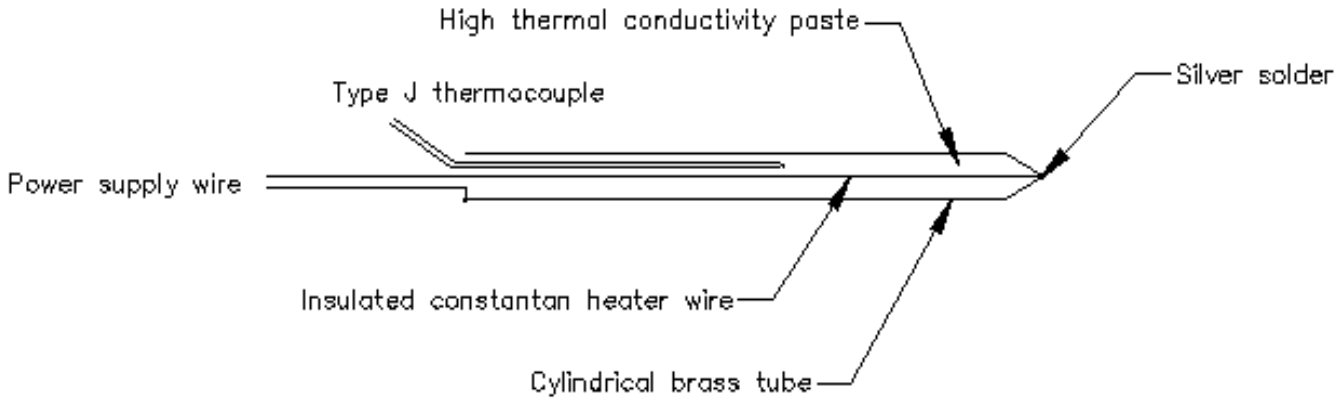


Figure B.1 Schematic diagram of the thermal conductivity probe.

B.3.3.2 Calibration and Error Determination

Based on experimental set up, there is always assumption that the idealized condition may be deviated (e.g. volume of heat source, filling materials inside the probe, etc.). To account for this deviation, a probe constant C was calibrated against reference materials of known thermal conductivity (distilled water and distilled water containing agar (1% w/v)). The value of this parameter is dependent on both reference material and probe (Wang and Hayakawa 1993). The empirical result of equation B.2 may be modified as follows (Lobo and Cohen 1990):

$$T_2\text{-}T_1 = \frac{CQ'}{4\pi k}\ln\left(\frac{t_2}{t_1}\right) \tag{B.3}$$

This may further transform as:

$$k = \frac{CQ'}{4\pi(T_2\text{-}T_1)}\ln\left(\frac{t_2}{t_1}\right) \tag{B.4}$$

Subsequently,

$$k = Ck' \tag{B.5}$$

C = calibration coefficient

k = thermal conductivity of the reference materials, and

k' = thermal conductivity of the measured distilled water and distilled water containing agar (1% w/v), and

$$k' = \frac{Q'}{4\pi(T_2 - T_1)} \ln\left(\frac{t_2}{t_1}\right) \tag{B.6}$$

The ratio of the thermal conductivity of the reference material to that of thermal conductivity of the measured distilled water and distilled water containing agar (1% w/v) gave the calibration coefficient of the probe.

Thermal conductivity of distilled water and distilled water containing agar (1% w/v) were measured at temperatures of 22°C, 25°C, and 90°C and compared with existing values reported in references to calculate the percentage error, as explained by Fontana et al. (2001), using equation B.7:

$$\%\text{error} = \frac{|k_R - k_m|}{k_R} \tag{B.7}$$

where k_R is thermal conductivity from reference and k_m is measured thermal conductivity.

B.3.4 Experimental Procedure

Thermal conductivity was measured at three levels of temperature (4°C, 25°C, and 90°C), five levels of the ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8), and three levels of hammer mill screen size (0.354, 0.8, and 1.6 mm) in three replicates. Each sample mixture was loaded into a glass container (30 mm wide and 90 mm height). The probe was inserted vertically down into the middle of the sample. To measure at higher temperature (90°C), the sample

mixture with inserted probe was put into a controlled environmental chamber (B-M-A Inc., Ayer, MA.) and used as external heat source Figure B.2. The sample and the probe were allowed to reach thermal equilibrium with the surrounding environment. The sample thermal conductivity was measured for about 1 min using the probe by applying a small constant voltage to the probe heater. Time and temperature were recorded in 0.125 s intervals using a Campbell data logger (model CR10X, Campbell Scientific, Inc., Logan, UT). A 20 min interval was allowed between tests so that the probe reached a stable thermal equilibrium. The power supply provided a constant current of 1.092 A. The heater wire resistance was measured to be 41.6 Ω m^{-1}.

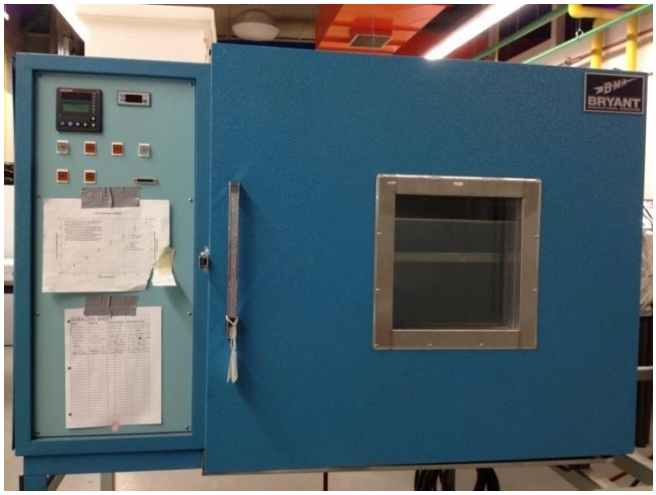


Figure B.2 Environmental chamber for heating biomass mixture during thermal conductivity measurement at 90^{o}C

The thermal conductivity value was calculated using the following equation:

$$k = \frac{I^2 R}{4\pi(T_2 - T_1)} \ln\left(\frac{t_2}{t_1}\right) \quad \text{(B.8)}$$

where I is the current (A) and R is the specific resistance of the heater wire (Ω m^{-1}). The local slope between 20 data point sequence was calculated from the natural logarithm of time and probe temperature using linear regression analysis, as shown in equation B.9:

$$S = \frac{T_2 - T_1}{\ln \frac{t_2}{t_1}} \quad \text{(B.9)}$$

where S is the slope. The thermal conductivity was determined by identifying the maximum slope, as explained by Wang and Hayakawa (1993), equation B.10:

$$k = \frac{I^2 R}{4\pi S} \quad \text{(B.10)}$$

B.3.5 Specific Heat Measurement

The specific heat of the samples was measured using a differential scanning calorimeter (DSC 2910, TA Instruments, New Castle, DE) with a liquid nitrogen cooling system (Figure B.3). The instrumental set-up was calibrated using a standard sample of sapphire. Specific heat was measured at three levels of temperature (70°C, 80°C, and 90°C), five levels of ratio of biomass: NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8), and three levels of hammer mill screen size (0.354, 0.8, and 1.6 mm) in three replicates. These temperatures are similar to the RF-alkaline pretreatment temperatures. The target is to compute the thermal diffusivity at 90°C. These temperatures are similar to the temperatures used for the RF-alkaline pretreatment. About 4 to 10 mg of sample was encapsulated within a flat, thin aluminum sample pan (approx. 6 mm). The sample pan and reference pan were heated up at the same heating rate of 5°C min^{-1}. The sample pan contained the measured material, as such; it requires more heat to be at the same temperature as the empty reference pan. Such extra heat flow was measured by the DSC. The specific heat was calculated by the TA Instruments analysis software (TA Instruments, New Castle, DE) based on heat flow and heating rate as shown in equation B.11 (Tang et al. 1991):

$$C_p = \frac{\frac{\Delta q}{t}}{\frac{m\Delta T}{t}} \quad \text{(B.11)}$$

where C_p is specific heat, q is heat (J), t is time (s), m is the mass of sample (kg), and ΔT is the temperature change (°C). The precision of the DSC for specific heat measurement was 50 J kg^{-1} $°C^{-1}$.

Figure B.3 Differential scanning calorimeter (DSC 2910).

B.3.6 Thermal Diffusivity Calculation

Thermal diffusivity was calculated by the indirect method as reported by Singh and Heldman (2009) and Yang et al. (2002) using equation B.1. Since thermal diffusivity was obtained as a secondary quantity, the uncertainty in the estimation of thermal diffusivity is dependent on the uncertainties related to the measured primary quantities (thermal conductivity, specific heat, and bulk density). Therefore, the error of measurement or uncertainty of thermal diffusivity ($G\mathfrak{O}_\alpha$) needs to be calculated using equation B.12, as reported by Ma et al. (1998) and Huggins (1983):

$$\omega_\alpha = \sqrt{\left(\frac{\partial_\alpha}{\partial_k}\omega_k\right)^2 + \left(\frac{\partial_\alpha}{\partial_{\rho_b}}\omega_{\rho_b}\right)^2 + \left(\frac{\partial_\alpha}{\partial_{C_p}}\omega_{C_p}\right)^2} \qquad \text{(B.12)}$$

where ω_k, ω_{ρ_b}, and ω_{C_p} are the error or uncertainties of thermal conductivity, bulk density, and specific heat, respectively, obtained from repeated measurements of the primary quantities (twice the standard deviation). Replacing each of the error terms with the following:

$$\frac{\partial_\alpha}{\partial_k}\omega_k = \frac{1}{\rho C_p}\omega_k \qquad \text{(B.13)}$$

$$\frac{\partial_\alpha}{\partial_{\rho_b}}\omega_{\rho_b} = \frac{k}{\rho^2 C_p}\omega_{\rho_b} \quad \text{(B.14)}$$

$$\frac{\partial_\alpha}{\partial_{C_p}}\omega_{C_p} = \frac{k}{\rho C^2{}_p}\omega_{C_p} \quad \text{(B.15)}$$

The substitution of equations B.13-B.15 into B.12 results:

$$\omega_\alpha = \sqrt{\left(\frac{1}{\rho C_p}\omega_k\right)^2 + \left(\frac{k}{\rho^2 C_p}\omega_{\rho_b}\right)^2 + \left(\frac{k}{\rho C^2{}_p}\omega_{C_p}\right)^2} \quad \text{(B.16)}$$

The error of measurement or uncertainty of the thermal diffusivity (ω_α) was calculated at 90°C, three levels of hammer mill screen size (0.354, 0.8, and 1.6 mm), and five levels of ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8).

B.3.7 Statistical Analysis

Experimental data were statistically analyzed via analysis of variance (ANOVA) using simple linear regression model. It was conducted at 5% significance level using IBM SPSS Statistics (Superior Performing Statistical Software, version 20 for Windows, 2012; IBM, Armonk, New York, NY).

B.4 Results and Discussion

B.4.1 Physical Characteristics of Lignocellulosic Biomass

Table B.1 shows the physical characteristics of lignocellulosic barley straw grind. As the hammer mill screen size decreased, the particle and bulk densities increased, while the inter-particle porosity increased with increased screen size but not significantly affected. Materials ground with smaller screen size have more compact particle arrangements with higher densities (Singh and Heldman 2009). There may also be some effect of moisture transfer and compaction during the grinding process that may be responsible for the change and differences in the particle

density of the different hammer mill screen size. Adapa et al. (2011) investigated the grinding performance and physical properties of non-treated and steam exploded barley, canola, oat, and wheat straw. The authors reported that the particle density of non-treated and steam exploded barley, canola, oat, and wheat straw significantly increased with a decrease in hammer mill screen size from 30 to 1.6 mm. Mani et al. (2004) performed a similar investigation on the grinding performance and physical properties of wheat and barley straws, corn stover, and switchgrass. These researchers reported a similar trend of particle density with respect to the hammer mill screen size.

Table B.1 Physical attributes of lignocellulosic barley straw grind

Physical characteristics	Hammer mill screen size		
	0.354 mm	0.8 mm	1.6 mm
Particle density (kgm^{-3})	1323.22 (1.94)	1272.72 (0.59)	1126.57 (2.14)
Bulk density (kgm^{-3})	130.36 (0.89)	124.75 (0.19)	98.35 (0.76)
Inter-particle porosity (%)	90 (0.00)	90 (0.00)	91 (0.00)
Porosity within particle (%)	30 (0.00)	-	-
Total porosity (%)	120	-	-

Values in parentheses = standard error; number of replicates, n = 3

The void spaces in biological materials are described by determining the porosity, which is expressed as the volume not occupied by the solid material (Singh and Heldman 2009). The inter-particle porosity obtained from this study was about three times higher than the porosity within the particles. This implies that the voids within the particles were much smaller than the void spaces between the particles, which are dependent on the particle size.

B.4.2 Thermal Conductivity

Table B.2 depicts the thermal conductivity of distilled water and distilled water containing agar (1% w/v). The percentage error was calculated based on equation B.7. Both the measured and reference thermal conductivity increased as the temperature increased. Table B.2 also shows the calibration coefficient (C) calculated using equation B.5. Lobo and Cohen (1990) reported that the ideal value of the constant (C) is 1. The same authors reported that the calibration coefficient is dependent on the probe characteristics and have little or no temperature sensitivity. In this

present study, the constant value obtained was between the ranges of 0.918-0.991, as such, the probe was acceptable for the measurement of thermal conductivity.

Table B.3 shows the thermal conductivity of lignocellulosic biomass sample at three levels of temperature (4^oC, 25^oC, and 90^oC) and hammer mill screen size (0.354, 0.8, and 1.6 mm), and five levels of ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8). Thermal conductivity of biomass:NaOH solution mixture increased as the temperature increased. Temperature plays an important role in the conductivity of heat within biological materials. The analysis of variance performed on the obtained data showed that temperature has a significant effect ($P < 0.05$) on thermal conductivity of the mixture across all levels of ratio of biomass:NaOH solution and hammer mill screen size (Table B.6). Thermal conductivity is affected by atomic activity. At higher temperature, there is random atomic motion and vibration within the biomass sample. Higher atomic activity within the biomass results to higher ability to transfer heat energy and subsequenctly higher thermal conductivity. Therefore, temperature describes the range of speeds of a bunch of atoms together. Tabil et al. (2003) investigated the thermal conductivity of sugarbeet roots at various levels of temperature. These authors reported that thermal conductivity slightly increased with temperature. This direct linear relationship between temperature and thermal conductivity has been reported by many researchers, such as those reported for chickpea flour, isolated starch, and isolated protein (Emami et al. 2007), packed bed of *Podophyllum peltatum* rhizome particles (Izadifar and Baik 2007), sucrose gel (Renaud et aI. 1992), apple (Ramaswamy and Tung 1981), tomato paste (Drusas and Saravacos 1985), and granular starch (Drouzas and Saravacos 1988; Lan et al. 2000).

Table B.2 Thermal conductivity of distilled water and distilled water containing agar (1% w/v) at different temperatures

Temperature (^oC)	k_m [a]	k_R [b]	Reference	(%) Error	C_m
4*	-	0.5680	Singh and Heldman (2001)	-	
		0.5756 (0.01)	Emami et al. (2007)		
20**	-	0.5970	Singh and Heldman (2009)	-	
22*	0.6134 (0.002)	0.5970	Singh and Heldman (2001)	2.747	0.973
		0.6079 (0.01)	Emami et al. (2007)	0.905	0.991
25**	0.6600 (0.004)	0.6060	Singh and Heldman (2009)	8.911	0.918
40*	-	0.633	Singh and Heldman (2001)	-	
		0.6176 (0.01)	Emami et al. (2007)		
90**	0.739 (0.015)	0.6780	Singh and Heldman (2009)	8.997	0.918

[a] k_m = measured thermal conductivity (W m^{-1} $^oC^{-1}$). Value in parenthesis is standard error, n = 3
[b] k_R = thermal conductivity from reference (W m^{-1} $^oC^{-1}$).
* = distilled water containing agar (1% w/v)
** = distilled water
C_m = calibration coefficient calculated from the ratio of reference to measured thermal conductivity.

The effect of temperature (90^oC) on thermal conductivity seem to be higher at lower ratio of biomass:NaOH solution, especially with 0.354 and 0.8 mm hammer mill screen size that was used to grind the biomass. The higher the ratio of biomass:NaOH solution, the lower was the thermal conductivity of the sample mixture. At 25^oC, the thermal conductivity increased as the ratio of biomass:NaOH solution decreased at 0.354 and 0.8 mm hammer mill screen size. The analysis of variance performed shows that ratio of biomass:NaOH solution has a significant effect ($P < 0.05$) on thermal conductivity of the mixture, but not at all levels of screen size and temperature (Table B.6). Thermal conductivity did not substantially change with hammer mill screen size in the range studied. The results obtained depicts that hammer mill screen size has significant effect ($P < 0.05$) on thermal conductivity only at higher temperature, 90^oC (Table B.6). The overall thermal conductivity may have been affected by the chemical composition, interaction, and reaction between the biomass and NaOH solution. It can also be seen that temperature significantly affected ($P < 0.05$) the thermal conductivity of dried raw biomass. As

the temperature of the dried raw biomass increased the thermal conductivity correspondingly increased. At higher temperature (90^{o}C), the thermal conductivity of dried raw biomass increased as the hammer mill screen size increased. 1% NaOH solution had higher thermal conductivity than dried raw sample at all levels of temperature studied. Comparison of thermal conductivity of dried raw sample at 90^{o}C with biomass:NaOH solution mixture shows that the thermal conductivity value of dried raw sample was lower than that of the biomass and NaOH solution mixture. This shows that mixture of biomass and NaOH solution played a more important role and has a stronger effect on thermal conductivity than the dried raw biomass sample. This may be due to the higher thermal conductivity of water at lower ratio of biomass:NaOH solution.

Table B.3 Thermal conductivity (k) of biomass sample at different temperatures, screen size, and ratio of biomass to NaOH solution.

Biomass:NaOH solution ratio	Screen size (mm)	Control environment (4^{o}C, 98% RH)	Room temperature (25^{o}C)	90^{o}C temperature
		k (W m^{-1} °C^{-1})	k (W m^{-1} °C^{-1})	k (W m^{-1} °C^{-1})
1:4	0.354	0.564 (0.015)	0.596 (0.012)	0.897 (0.033)
1:4	0.8	0.548 (0.016)	0.596 (0.020)	0.862 (0.020)
1:4	1.6	0.523 (0.043)	0.602 (0.008)	0.766 (0.031)
1:5	0.354	0.542 (0.044)	0.642 (0.009)	0.889 (0.018)
1:5	0.8	0.558 (0.018)	0.618 (0.012)	0.944 (0.014)
1:5	1.6	0.596 (0.005)	0.556 (0.073)	0.722 (0.019)
1:6	0.354	0.618 (0.018)	0.658 (0.022)	0.995 (0.066)
1:6	0.8	0.582 (0.019)	0.623 (0.009)	0.950 (0.094)
1:6	1.6	0.577 (0.007)	0.626 (0.016)	0.695 (0.005)
1:7	0.354	0.621 (0.026)	0.691 (0.079)	0.861 (0.058)
1:7	0.8	0.640 (0.009)	0.635 (0.031)	1.137 (0.021)
1:7	1.6	0.629 (0.009)	0.557 (0.003)	0.714 (0.013)
1:8	0.354	0.607 (0.021)	0.631 (0.014)	1.332 (0.121)
1:8	0.8	0.631 (0.016)	0.653 (0.006)	1.013 (0.021)
1:8	1.6	0.617 (0.016)	0.658 (0.0486)	0.755 (0.016)
Raw sample	0.354	0.641 (0.019)	-	0.845 (0.018)
Raw sample	0.8	0.602 (0.007)	-	0.811 (0.018)
Raw sample	1.6	0.641 (0.016)	-	0.848 (0.023)
1% NaOH	-	0.730 (0.002)	-	1.935 (0.468)

RH = relative humidity; value in parenthesis is standard error; n = 3

B.4.3 Specific Heat

The specific heat of the lignocellulosic biomass was determined at three levels of temperature (70^oC, 80^oC, and 90^oC) and hammer mill screen size (0.354, 0.8, and 1.6 mm), and five levels of ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8). Depending on the temperature, ratio of biomass:NaOH solution, and hammer mill screen size, the specific heat values ranged from 2.485 to 3.976 kJ $kg^{-1}°C^{-1}$. At higher ratio of biomass:NaOH solution (1:4), the specific heat increased as the temperature increased from 70^oC to 90^oC at all levels of hammer mill screen size. At ratio 1:5, specific heat increased as the temperature increased from 70^oC to 80^oC and slightly decreased at 90^oC at the three hammer mill screen size studied. While at lower ratios (1:6-1:8), the specific heat decreased as the temperature increased from 70^oC to 90^oC at all levels of hammer mill screen size. This implies that less energy/heat is required to increase the temperature of the mixture after it has been heated up from the ground temperature (revise, not clear). This could also be attributed to the higher thermal conductivity of liquid (1% NaOH solution) present in the mixture. It also reflects the higher thermal conductivity of the mixture at lower temperature as reported above in section 3.2. The specific heat of the mixture did not change significantly with the ratio of biomass:NaOH solution in the range studied at all levels of hammer mill screen size and temperature. The specific heat at the five ratios studied seemed to decrease as the temperature increased. This may be associated with the chemical reactions which start or accelerated at higher temperatures. Statistical analysis shows that the ratio of biomass:NaOH solution has significant effect ($P < 0.05$) on the specific heat of the mixture only at 90^oC and 0.354 mm hammer mill screen size (Table 6). This study shows that hammer mill screen size does not have any significant effect ($P > 0.05$) on the specific heat of biomass:NaOH mixture at all levels of temperature and ratio of biomass:NaOH solution. However, hammer mill screen size has significant effect ($P < 0.05$) on the specific heat of dried raw biomass. The specific heat of dried raw biomass sample increased as the hammer mill screen size increased from 0.354 to 1.6 mm and as temperature increased from 70^oC to 90^oC. This direct linear relationship between hammer mill screen size and specific heat of the dried raw biomass can be attributed to difference in density resulting from the pores and air pockets within the biomass particles. More energy is required in heating up the pores/air (with higher porosity and lower bulk density). This ultimately results to higher specific heat. This implies that smaller hammer

mill screen size will require less energy during the pretreatment (heating) process. However, there is a lot of energy consumption in further reduction of hammer mill screen size from 1.6 mm to 0.354 mm. Iroba and Tabil (2013a) reported that costs and energy requirements for particle size reduction increases geometrically with decreasing particle size. Iroba et al. (2013) investigated the effect of radio frequency-assisted alkaline pretreatment of barley straw. These authors demonstrated that there was no significant difference between the acid soluble lignin moieties and insoluble lignin obtained from biomass ground using hammer mill at 0.8 and 1.6 mm screen size. As such, these researchers adopted 1.6 mm screen size for their investigation.

Table B.4 Specific heat (C_p) of biomass sample at different temperatures, screen size, and ratio of biomass:NaOH solution

Biomass:NaOH solution ratio	Screen size (mm)	Temperature (^oC)		
		70	80	90
		C_p (kJ $kg^{-1}°C^{-1}$)	C_p (kJ $kg^{-1}°C^{-1}$)	C_p (kJ $kg^{-1}°C^{-1}$)
1:4	0.354	3.377 (0.146)	3.439 (0.162)	3.467 (0.261)
1:4	0.8	3.516 (0.689)	3.779 (0.922)	2.887 (0.097)
1:4	1.6	2.873 (0.478)	2.903 (0.487)	2.951 (0.471)
1:5	0.354	3.882 (0.435)	3.976 (0.658)	3.639 (0.363)
1:5	0.8	3.190 (0.297)	3.429 (0.447)	3.320 (0.316)
1:5	1.6	3.208 (0.365)	3.266 (0.391)	3.212 (0.437)
1:6	0.354	3.251 (0.276)	3.202 (0.282)	3.125 (0.306)
1:6	0.8	3.339 (0.196)	3.212 (0.230)	3.026 (0.116)
1:6	1.6	3.579 (0.370)	3.468 (0.420)	3.314 (0.457)
1:7	0.354	3.392 (0.154)	3.849 (0.586)	3.167 (0.055)
1:7	0.8	3.819 (0.148)	3.657 (0.176)	3.535 (0.232)
1:7	1.6	3.429 (0.394)	3.214 (0.495)	3.088 (0.542)
1:8	0.354	3.361 (0.188)	3.103 (0.308)	2.592 (0.232)
1:8	0.8	2.858 (0.365)	2.485 (0.677)	3.288 (0.418)
1:8	1.6	3.366 (0.392)	3.185 (0.430)	3.022 (0.471)
Raw sample	0.354	0.868 (0.500)	0.821 (0.543)	0.829 (0.573)
Raw sample	0.8	1.805 (0.192)	1.832 (0.205)	1.890 (0.211)
Raw sample	1.6	2.686 (0.775)	2.757 (0.877)	2.856 (0.953)
1% NaOH	-	1.111 (0.253)	0.434 (0.221)	0.331 (0.119)

Value in parenthesis is standard error; n = 3

Mani et al. (2004) investigated the grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass, and concluded that the grinding energy consumption increases as the biomass particle size becomes finer. In this present study, temperature has

significant effect ($P < 0.05$) on specific heat of the dried raw biomass only at ratio 1:8 at 0.354 mm screen size. The specific heat of dried raw biomass sample decreased as the temperature increased at 0.354 mm hammer mill screen size. However, the specific heat increased as the temperature increased from 70°C-90°C at 0.8 and 1.6 mm hammer mill screen size. Li et al. (2008) investigated the specific heat of flax fiber-HDPE biocomposites at processing temperatures (170°C-200°C). These authors reported that the specific heat of the biocomposites increased slowly with temperature. Izadifar and Baik (2007) demonstrated that the specific heat of packed bed of *Podophyllum peltatum* rhizome particles increases with increasing temperature. Emami et al. (2007) also reported that the specific heat of chickpea flour, isolated starch, and isolated protein increased with temperature.

In this study, the specific heat value of 1%NaOH decreased as the temperature increased from 70°C to 90°C. This also reflects the good thermal conductivity of liquids (1% NaOH solution) at higher temperature that requires less energy/heat to increase the temperature.

B.4.4 Thermal Diffusivity

The thermal diffusivity values of the biomass sample was calculated at 90°C, using three levels of hammer mill screen size (0.354, 0.8, and 1.6 mm), and five levels of ratio of biomass:NaOH solution (1:4, 1:5, 1:6, 1:7, and 1:8) with the corresponding uncertainty or error of estimation ($GƆ_{\alpha}$). Thermal diffusivity of the biomass:NaOH solution mixture and the dried biomass sample did not show any specific trend with the ratio of biomass:NaOH solution, as well as with the hammer mill screen size (with different bulk density). At higher ratio of biomass:NaOH solution (1:4 and 1:5), thermal diffusivity decreased with increasing bulk density from 98.35 kg m^{-3} to 130.36 kg m^{-3} and decreasing hammer mill screen size from 1.6 mm to 0.354 mm. The same trend was observed at lower ratio of biomass:NaOH solution (1:6 and1:7) but only at hammer mill screen size of 0.354 mm and 0.8 mm, and decreased at 1.6 mm. This may be attributed to the chemical reaction between the biomass particles and NaOH solution. Emami et al. (2007) calculated the thermal diffusivity of chickpea flour, isolated starch, and isolated protein. These authors reported that the thermal diffusivity of these biological materials decreased with an increase in bulk density. The thermal diffusivity of dried raw biomass sample did not follow a

specific trend across the three hammer mill screen size studied. The highest overall thermal diffusivity was obtained from the dried raw biomass sample at 0.354 mm, followed by ratio 1:8 of biomass:NaOH solution at the same screen size of 0.354 mm. At higher bulk density (hammer mill screen size of 0.354 mm), the porosity within and between the dried raw biomass particles are minimal, hence, there will be higher and ease for thermal diffusivity within the bulk of biomass particles, as shown in equation B.1 above. Emami et al. (2007) and Lan et al. (2000) also reported a similar random relationship of thermal diffusivity of biological materials with the investigated parameter (temperature).

Table B.5 Thermal diffusivity (α) of biomass sample at different hammer mill screen size, ratio of biomass to NaOH solution, and at 90^{o}C

Biomass: NaOH solution ratio	Thermal diffusivity, α (10^{-3} m^2 s^{-1})					
	0.354 mm		0.8 mm		1.6 mm	
	ρ_b (kg $m^{-3)}$ = 130.36	$Ↄ_\alpha$ (10^{-3})	ρ_b (kg $m^{-3)}$ = 124.75	$Ↄ_\alpha$ (10^{-3})	ρ_b (kg $m^{-3)}$ = 98.35	$Ↄ_\alpha$ (10^{-3})
1:4	2.015 (0.215)	3.110	2.455 (0.113)	1.650	2.757 (0.371)	3.538
1:5	1.916 (0.213)	1.862	2.318 (0.202)	1.530	2.381 (0.352)	2.420
1:6	2.502 (0.326)	5.550	2.529 (0.300)	7.923	2.212 (0.292)	1.198
1:7	2.087 (0.146)	4.920	2.604 (0.196)	2.187	2.495 (0.409)	1.998
1:8	4.124 (0.076)	8.445	2.490 (0.314)	2.217	2.644 (0.332)	2.147
Raw sample	18.714 (8.640)	18.712	3.511(0.313)	1.638	3.678 (1.001)	4.197

ρ_b = bulk density
$Ↄ_\alpha$ = the error of measurement or uncertainty of thermal diffusivity
value in parenthesis is standard error (10^{-3}), n = 3

Table B.6 Analysis of variance determining the effect of independent variables (temperature, biomass:NaOH solution ratio, and hammer mill screen size) on the dependent variable (thermal conductivity and specific heat)

Thermal conductivity, k	P-value	Specific heat, C_p	P-value
Effect of hammer mill screen size @		Effect of hammer mill screen size @:	
Ratio 1:4 @ 4°C	0.29	Ratio 1:4 @ 70°C	0.41
Ratio 1:4 @ 25°C	0.77	Ratio 1:4 @ 80°C	0.46
Ratio 1:4 @ 90°C	0.01	Ratio 1:4 @ 90°C	0.36
Ratio 1:5 @ 4°C	0.17	Ratio 1:5 @ 70°C	0.29
Ratio 1:5 @ 25°C	0.17	Ratio 1:5 @ 80°C	0.36
Ratio 1:5 @ 90°C	0.01	Ratio 1:5 @ 90°C	0.44
Ratio 1:6 @ 4°C	0.14	Ratio 1:6 @ 70°C	0.40
Ratio 1:6 @ 25°C	0.28	Ratio 1:6 @ 80°C	0.52
Ratio 1:6 @ 90°C	0.01	Ratio 1:6 @ 90°C	0.62
Ratio 1:7 @ 4°C	0.83	Ratio 1:7 @ 70°C	0.92
Ratio 1:7 @ 25°C	0.15	Ratio 1:7 @ 80°C	0.31
Ratio 1:7 @ 90°C	0.20	Ratio 1:7 @ 90°C	0.76
Ratio 1:8 @ 4°C	0.80	Ratio 1:8 @ 70°C	0.84
Ratio 1:8 @ 25°C	0.52	Ratio 1:8 @ 80°C	0.78
Ratio 1:8 @ 90°C	<0.01	Ratio 1:8 @ 90°C	0.40
Raw dried sample @ 4°C	0.80	Raw dried sample @ 70°C	0.04
Raw dried sample @ 90°C	0.76	Raw dried sample @ 80°C	0.05
		Raw dried sample @ 90°C	0.06
Effect of ratio of biomass:NaOH solution @		Effect of ratio of biomass:NaOH solution @:	
0.354 mm @ 4°C	0.08		
0.354 mm @ 25°C	0.33		
0.354 mm @ 90°C	0.02	0.354 mm @ 70°C	0.54
0.8 mm @ 4°C	<0.01	0.354 mm @ 80°C	0.57
0.8 mm @ 25°C	0.02	0.354 mm @ 90°C	0.01
0.8 mm @ 90°C	0.01	0.8 mm @ 70°C	0.59
1.6 mm @ 4°C	<0.01	0.8 mm @ 80°C	0.18
1.6 mm @ 25°C	0.48	0.8 mm @ 90°C	0.36
1.6 mm @ 90°C	0.69	1.6 mm @ 70°C	0.32
		1.6 mm @ 80°C	0.70
		1.6 mm @ 90°C	0.99

Effect of temperature @:		Effect of temperature @:	
Ratio 1:4 @ 0.354 mm	<0.01	Ratio 1:4 @ 0.354 mm	0.74
Ratio 1:4 @ 0.8 mm	<0.01	Ratio 1:4 @ 0.8 mm	0.49
Ratio 1:4 @ 1.6 mm	<0.01	Ratio 1:4 @ 1.6 mm	0.90
Ratio 1:5 @ 0.354 mm	<0.01	Ratio 1:5 @ 0.354 mm	0.74
Ratio 1:5 @ 0.8 mm	<0.01	Ratio 1:5 @ 0.8 mm	0.79
Ratio 1:5 @ 1.6 mm	0.05	Ratio 1:5 @ 1.6 mm	0.99
Ratio 1:6 @ 0.354 mm	<0.01	Ratio 1:6 @ 0.354 mm	0.75
Ratio 1:6 @ 0.8 mm	<0.01	Ratio 1:6 @ 0.8 mm	0.24
Ratio 1:6 @ 1.6 mm	<0.01	Ratio 1:6 @ 1.6 mm	0.64
Ratio 1:7 @ 0.354 mm	0.02	Ratio 1:7 @ 0.354 mm	0.68
Ratio 1:7 @ 0.8 mm	<0.01	Ratio 1:7 @ 0.8 mm	0.29
Ratio 1:7 @ 1.6 mm	0.12	Ratio 1:7 @ 1.6 mm	0.61
Ratio 1:8 @ 0.354 mm	<0.01	Ratio 1:8 @ 0.354 mm	0.03
Ratio 1:8 @ 0.8 mm	<0.01	Ratio 1:8 @ 0.8 mm	0.71
Ratio 1:8 @ 1.6 mm	<0.01	Ratio 1:8 @ 1.6 mm	0.56
Raw dried sample @ 0.354 mm	<0.01	Raw dried sample @ 0.354 mm	0.96
Raw dried sample @ 0.8 mm	<0.01	Raw dried sample @ 0.8 mm	0.76
Raw dried sample @ 1.6 mm	<0.01	Raw dried sample @ 1.6 mm	0.89

B.5 Conclusion

Temperature plays an important role in the conduction of heat within biological materials. Thermal conductivity of biomass:NaOH solution mixture increased linearly with temperature. The effect of ratio of biomass:NaOH solution on thermal conductivity of the mixture was observed at higher temperature (90^{o}C). Specific heat of the biomass:NaOH solution mixture did not change significantly with hammer mill screen size in the range studied. At higher ratio of biomass:NaOH solution, the specific heat increased linearly with temperature. While at lower ratio, the specific heat decreased as the temperature increased. 1% NaOH solution has higher thermal conductivity with lower specific heat as compared to the dried raw biomass and biomass:NaOH solution mixture. Thermal diffusivity of the biomass:NaOH solution mixture and the dried biomass sample did not show any specific trend with the ratio of biomass:NaOH solution, as well as with the hammer mill screen size (with different bulk density).

Appendix C

C. Radio Frequency-Alkaline Pretreatment of Lignocellulosic Barley Straw

A similar version of this chapter has also been presented and published in the International conference of Canadian Society of Biological Engineers:

- Iroba, K.L., L.G. Tabil, T. Canam and T. Dumonceaux. 2011. Radio frequency-alkaline pretreatment of lignocellulosic barley straw. CSBE/SCGAB 2011 Annual Conference, Paper No. CSBE11-306, Inn at the Forks, Winnipeg, Manitoba, 10-13 July.

C.1 Abstract

The lignocellulosic nature of biomass presents resistance and 'recalcitrance' to the biological and chemical degradation of the lignocellulosic materials during enzymatic hydrolysis or saccharification, and the subsequent fermentation process. This leads to very low conversion rate, which makes the process economically infeasible. In this study, radio-frequency (RF)-based dielectric heating technique was used in the alkaline (NaOH) pretreatment of lignocellulosic biomass barley straw, so as to enhance its accessibility and digestibility by enzymatic reaction during hydrolysis. Due to the inherent and unique features of RF heating, samples can be pretreated under conditions which might not be conducive for microwave or the conventional heating methods. Two levels of sample particle size (1.6 and 0.8 mm), three levels of temperature (40, 50, and 60^{o}C), 1 h soaking time, and two levels of NaOH concentration (0.5 and 1%) at 20 min residence time were used for the radio frequency pretreatment. The effect of the RF pretreatment was assessed through chemical composition analysis and densification of the pretreated and non-treated biomass samples. Statistical analysis showed that 1% NaOH

concentration has significant effect on the average acid insoluble lignin, ash content, and the physical characteristics of the produced pellets.

C.2 Introduction

The rise in the atmospheric CO_2 level has spurred up worldwide attention. There is a general acceptance within the scientific community that the emission greenhouse gas (GHG) is responsible for the global climate change (forced warming), which is mainly from the combustion of fossil fuels (Fiona et al. 2007). These energy sources are not renewable because it takes millions of years to be formed. There is fear of depletion of petroleum, coupled with the geometric increasing world population. As part of the strategies to mitigate the above negative effects; reduce the carbon footprint, and also enhance sustainability of energy supply, bioenergy and biofuels are espoused with lignocellulosic biomass as feedstock which is readily available, renewable, and carbon sink source of energy (Demirbas et al. 2009). Lignocellulosic biomass has an annual production of approximately 200 billion tonnes worldwide (Zhang 2008). Lignocellulosic biomass has long been known as a potential sustainable source of reduced sugars for fermentation to produce biofuels and other biomaterials. This will help to address climate change and create a healthier environment for today and tomorrow, avoid the competition between food and energy, create job opportunities, and generate revenue for the governments both at federal and provincial levels. Lignocellulosic biomass is a complex formation of cellulose, hemicellulose, and lignin (Fan et al. 2006a). The lignin (deposited at maturity) acts as an external cross-linked aromatic polymer based on phenylpropanoid units surrounding hemicellulose and cellulose with cellulose positioned at the inner core of the structure (Fan et al. 2006a; Lin and Tanaka 2006). Hemicellulose has a random, branched, and amorphous structure with little strength compared to cellulose (Lin and Tanaka 2006). Cellulose is a polysaccharide with crystalline, amorphous, strong structure, and high degree of polymerization (Fan et al. 2006a; Lin and Tanaka 2006). Cellulose and hemicellulose have the potential for cellulosic bioethanol production. This lignocellulosic binding nature presents resistance and ‘recalcitrance’ to the biological and chemical degradation of the lignocellulosic materials during saccharification/enzymatic hydrolysis and the subsequent fermentation process. This leads to very low conversion rate, which makes the process economically infeasible (Fan et al. 2006a;

Chandra et al. 2007; Mohammad and Karimi 2008). As such, initial pretreatment is required on the biomass prior to enzymatic hydrolysis.

C.2.1 Radio Frequency Technique

Radio frequency (RF) has been successfully used in drying (Balakrishnan et al. 2004) and thermal therapy (Maurizio et al. 2004). Application of RF heating has been reported in other research fields such as food processing (blanching, tempering, pasteurization, sterilization) and medicine (Punidadas et al. 2003). In recent time, Izadifar et al. (2009) demonstrated that RF can be used for the extraction of podophyllotoxin from rhizomes of *P. peltatum*. In RF heating, there is a volumetric heat generation inside the product which is a result of the interaction between the RF waves and the molecules of the product, unlike conventional method where heat is being transferred from the heating medium to the product (conduction or convection). Microwave (MW) heating has the same principles and mechanisms of heating as RF heating. In RF and MW, heating is based on the material's ability to absorb electromagnetic radiation and convert it into heat (Ryynanen 1995). However, RF heating has the following advantages over the MW heating: uniform electric field strength within the chambers, as such avoiding thermal runaway, large penetration depth (10-30 meters), and higher energy efficiency (Punidadas et al. 2003; Ryynanen 1995; Ramaswamy and Tang 2008). RF band has range of frequency between 3 kHz to 300 MHz, but typically 13.56-27.12 MHz and 40.68 MHz are used. The MW has 300 MHz to 300 GHz, typically 915 MHz, 2,450 MHz, 5.8 GHz, and 24.124 GHz are used (Ryynanen 1995; Punidadas et al. 2003). These ranges of frequency are selected and used for domestic, industrial, scientific, and medical applications so as to avoid interference with communication systems (Ryynanen 1995; Punidadas et al. 2003). RF heating can easily be scaled up.

C.2.2 Densification

In its natural form, most lignocellulosic biomass are bulky, loose, and disperse, as such, biomass are difficult to utilize as a fuel (Mani et al. 2006a). Hence, the large volume requirements of biomass as a feedstock for biorefinery does not present easy, economical, and efficient transportation, handling and storage characteristics (Tabil 1996; Mani et al. 2006a; Wooley et al. 1999). To avoid this unwanted condition is where densification gains extreme attention and

importance. Biomass densification may be described as the compaction or compressions of biomass to remove any form of inter or intra particle void/empty spaces within the material. Densification of non-treated biomass feedstock is difficult and results to production of pellets with poor physical quality rating. Lignocellulosic biomass has a natural binder (lignin), this act as an adhesive/glue that binds the biomass particles during the densification process (Granada et al. 2002). To manufacture pellets with good quality that could withstand the shear, tumbling, impact and rotation during transportation requires the released of this natural binder from the lignocellulosic matrix. Therefore, there is a need to fully or partially break (pretreatment) the lignocellulosic matrix in the biomass before densification so as to have access to lignin, and subsequently produce good durable pellets

No study has been reported on the use of RF heating as a pretreatment method on lignocellulosic biomass barley straw. The possible applicability of RF as a pretreatment method on lignocellulosic biomass was confirmed in this research. The RF technique was applied using NaOH catalyst. The effect of the RF pretreatment was assessed through chemical composition analysis and densification of the pretreated and non-treated biomass samples.

C.3 Methods and Procedures

C.3.1 Sample Procurement and Preparation

Lignocellulosic barley straw was obtained in October, 2009 from RAW Ag Ventures Limited, Maymont, SK. The straw was ground using a laboratory hammer mill (Serial no. GM13688; Glen Mills Inc., Maywood, NJ). Hammer mill screen sizes of 0.8 and 1.6 mm were used to grind the biomass straw. A dust collector (House of Tools, Model no. DC-202B, Saskatoon, SK) was connected to the outlet of the hammer mill to control dust during operation and to provide flow of the biomass straw in and out of the hammer mill. The initial moisture content of the straw was 8.8% (d.b.). The moisture content was measured based on ASABE standard method, ASAE S358.2 DEC1988 (R2008).

C.3.2 Particle and Bulk Density Measurement

The particle density of the samples was measured using the gas multi-pycnometer (QuantaChrome, Boynton Beach, FL 33426, model No. MVP-2) by calculating the displaced volume of nitrogen gas by a known mass of material. Three replicate tests were performed on each sample. The bulk density of the samples was determined by passing the material through a funnel which was placed above a standard 0.5 liter steel cup (SWA 951, Superior Scale Co. Ltd., Winnipeg, MB). Blockages in the funnel during biomass straw flow were cleared using a thin steel rod. After filling the cup, the excess was removed by passing a steel roller in a zig-zag manner over the top of the cup. The mass contained in the cup was then determined. Bulk density was calculated by dividing the mass of material within the cup by the volume of the cup. Three replicates were performed for each sample. The inter particle porosity (*Øip*) of the ground samples was computed from the particle and bulk densities (Table C.1) using equation C.1.

$$\phi_{ip} = 1 - \frac{\rho_b}{\rho_p} \qquad \text{(C.1)}$$

Where ρ_b is bulk density and ρ_p is particle density.

C.3.3 Radio Frequency Pretreatment

The pretreatment was performed using RF machine (1.5 kW and 27.12 MHz laboratory dryer, Strayfield, Theale, Reading) with the following process and material variables: three levels of temperature (40, 50, and 60^{o}C), ratio 1:6 (35 g of biomass and 210 g of NaOH solution), biomass particle size of 0.8 and 1.6 mm, using 0.5 and 1% NaOH concentration, and 1 h soaking time. The biomass samples and the NaOH solution were properly mixed together in Lexan polycarbonate reactor, with 1 h soaking time at room temperature before subjecting the mixture to RF pretreatment. When the temperature gets to the preset point, 20 min retention time was given for the mixture to heat up. Each treatment combination was duplicated. Three fiber optic temperature sensors were connected to the reactor at three different locations to monitor the heat distribution (temperature profile not shown in this paper), see Figure C.1. The pretreated samples were dried to 10% moisture (w.b.) using thin layer dryer at 40^{o}C. To evaluate the efficiency of

the pretreatment, chemical composition analysis and densification of the pretreated samples were carried out.

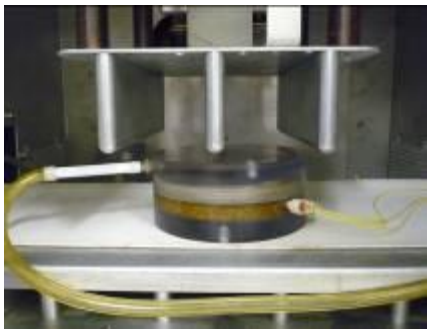


Figure C.1 Lexan polycarbonates reactor containing the biomass NaOH mixture inserted between two electrodes of the RF machine.

C.3.4 Chemical Composition Analysis

The National Renewable Energy Laboratory (NREL) standard (Sluiter et al. 2007) was used for the chemical composition analysis. Prior to this analysis, the biomass straw was bone dried at 105^{o}C. The non-structural carbohydrates (nitrites, protein, ash, waxes, chlorophyll, etc.) were removed using acetone extraction process. The NREL standard uses a two-step acid (72 and 4% H_2SO_4) hydrolysis to fractionate the biomass into forms that are more easily quantified. The lignin fractionates into acid insoluble material and acid soluble material. The acid insoluble lignin (AIL) was calculated using equation C.2 and the acid soluble lignin (ASL) was measured by UV-Vis spectroscopy and subsequently calculated using equation C.3.

$$AIL = \frac{(dried\_retentate)}{(dried\_sample)} \times 100\% \tag{C.2}$$

$$\%ASL = \frac{UV_{abs} \times Volume_{filtrate} \times Dilution\_factor}{\varepsilon \times M_{sample} \times specpathlength} \times 100\% \tag{C.3}$$

Where UV_{abs} is the average UV-Vis absorbance for the sample at 240 nm, ε is feedstock absorptivity constant (110 $Lg^{-1}cm^{-1}$), M_{sample} is the dried sample mass (0.3 g), $Volume_{filtrate}$ is volume of filtrate (87 mL), and dilution_factor is the dilution factor of the sample (10). During the hydrolysis the polymeric carbohydrates (cellulose and hemicellulose) were hydrolyzed into monomeric forms (xylose, arabinose, glucose, galactose, and mannose), which are soluble in the

hydrolysis liquid. These were measured using UPLC (Acquity 2004-2010, Waters Corporation, Milford, MA). The lignin piece and furfurals (5-hydroxyl-methyl furfural and furfural) were also measured using the UPLC. The chemical analysis was done using 300 ± 10 mg of each of the dried pretreated and untreated biomass straw.

C.3.5 Densification of Pretreated and Non-Treated Samples

The RF pretreated and non-treated samples were densified using a single pelleting Instron machine. This unit includes a steel cylindrical die with a plunger/piston which is connected to the upper moving crosshead to provide the load needed to compress the biomass samples. The die is surrounded with a heating element to provide the required heat for the process. About 0.5-0.7 g of the biomass feedstock was loaded into the die cylinder. The pre-set load (4 kN) and the required temperature (95±0.3^{o}C) are adjusted to compress the charge material. Once the pre-set load is attained, the plunger stops and held in position for 60 s retention time to avoid spring-back effect of biomass (Mani et al. 2006a), thereafter, the pellet is ejected. The Instron is typically set to lower the plunger (and compress the biomass) at a rate of 50 mm/min. The single-pelleter was connected to a computer which recorded the time and force-displacement data.

C.3.4.1 Durability Test

The durability or the hardness of the pellets was done after two weeks of storage, by dropping the pellets from a height of 1.85 m. The percentage durability was calculated by dividing the remaining piece by the original mass and multiplied by 100.

C.3.4.2 Tensile Strength

Diametral compression test was performed to evaluate the tensile strength of the biomass pellets (Tabil and Sokhansanj 1996) using the single pelleting Instron machine. Pellets were cut diametrically using a scalpel into tablets with thickness of about 2.21 mm. Single tablet was placed on its edge on the lower padded plate and compressed with 1000 N load cell by the upper plunger at a crosshead speed of 1 mm/min until failure occurred. Fractures that caused the tablets to break or crack in two halves along the loading axis were accepted and other fracture types were discarded (Tabil and Sokhansanj 1996). Upon failure, the fracture load/force was recorded

and the tensile strength of tablets was calculated using equation C.4. Ten replicates were made for each sample.

$$\delta_x = \frac{2F}{\pi dl} \quad \text{(C.4)}$$

Where δ_x is tensile (horizontal) stress (Pa), F the Load at fracture (N), d the tablet diameter (6.67 mm), and l the tablet thickness (m).

C.3.4.3 Pellet Density and Dimensional Stability

The mass, length, and diameter of the pellets were measured immediately after pelleting and after two weeks of storage. These parameters were used to calculate the density.

C.3.5 Statistical Analysis

Experimental data were statistically analyzed using linear regression analysis performed at 5% significance level using SPSS statistical software (version 14 for Windows, 2005 SPSS Inc).

C.4 Results and Discussion

Table C.1 depicts the physical characteristics of the untreated biomass ground. It shows that as the particle size decreases the particle and bulk densities increases, while porosity increases with increase particle size.

Table C.1 Physical characteristics of barley straw grind

Physical characteristics	Hammer mill screen size	
	0.8 mm	1.6 mm
Particle density (kg/m^3)	1272.72 (0.59)	1126.57 (2.14)
Bulk density (kg/m^3)	124.75 (0.19)	98.35 (0.76)
Porosity (%)	90 (0.00)	91 (0.00)

C.4.1 Chemical Composition Analysis

Table C.2 shows the chemical composition analysis of the RF pretreated and untreated samples. It shows that NaOH concentration is a major factor in the pretreatment. NaOH causes swelling

and creates pores on the biomass, separating the structural linkages between lignin and the complex carbohydrates (cellulose and hemicellulose), leading to increased surface area, and thus enhances the reactivity of the matrix with any external added material such as enzyme. The higher the NaOH concentration, the lower the acid insoluble lignin. The reason for this solid loss is because, during the pretreatment process, the ester bonds between lignin and the complex carbohydrates are disrupted (Sun and Cheng 2002; Feist et al. 1970), some lignin are broken down, degraded, and perhaps solubilized, and subsequently washed away during the extraction process which was performed prior to the chemical composition analysis. This shows the reason why the non-treated biomass has higher acid insoluble lignin. The implication of this is that the pretreated samples will have higher accessibility and digestibility during enzymatic reaction due to the already created pores. The higher the NaOH concentration the higher the ash content. 1% and 0.5% concentration increases the ash content by about 100 and 40%, respectively. This problem of increased ash content can be addressed by washing the pretreated samples. The statistical analysis performed shows that concentration has significant effect on the average acid insoluble lignin and ash content. There is no statistical difference observed among the average acid soluble lignin measured by UV-Vis spectroscopy. This may be attributed to the low temperatures used in this experiment, as well as the capability of the UV-Vis spectroscopy.

Table C.2 Chemical composition analysis of radio frequency pretreated barley straw grind

Tempt. (^{o}C)	NaOH conc. (%)	Hammer mill screen size (mm)	AIL (%)	ASL (%)	Ash content (%)	% ash increase
40	1	1.6	17.48 (0.69)	1.86 (0.49)	12.18 (0.31)	104.28
50	1	1.6	17.01 (1.29)	1.47 (0.07)	13.99 (0.10)	134.71
60	1	1.6	16.18 (1.45)	1.48 (0.00)	12.20 (0.04)	104.61
40	0.5	1.6	18.02 (1.12)	1.43 (0.05)	8.49 (0.10)	42.45
50	0.5	1.6	17.97(1.97)	1.55 (0.03)	8.54 (0.04)	43.19
60	0.5	1.6	18.69 (0.25)	1.94 (0.59)	8.67 (0.12)	45.38
40	1	0.8	17.19 (0.12)	1.44 (0.00)	11.85 (0.37)	98.77
50	1	0.8	16.38 (0.98)	1.55 (0.11)	12.01 (0.29)	101.36
60	1	0.8	16.82 (0.78)	1.46 (0.01)	11.76 (0.12)	97.24
24	1	0.8	19.05 (3.28)	1.50 (0.01)	11.78 (0.11)	97.55
24	0.5	0.8	18.18 (0.64)	1.49 (0.04)	8.39 (0.46)	40.71
24	0	0.8	19.50 (0.37)	1.52(0.03)	5.96 (0.40)	0.00

Values in parentheses = standard deviation; n = 2, Tempt. = temperature, Conc. = concentration.

Table C.3 depicts the average sum of 6 different lignin piece (4-hydroxybenzoic acid, vanillic acid, syringic acid, 4-hydroxybenzaldehyde, vanillin, syringaldehyde) and 2 furfurals (5-hydroxyl-methyl furfural (HMF), and furfural) analyzed using UPLC. It shows that the higher the NaOH concentration, the more pores are created on the lignin matrix, which makes it easy to access or solubilized the lignin component during the chemical composition analysis (two steps acid hydrolysis). The lignin in the raw sample is more difficult to solubilze during the chemical composition analysis, because the lignin matrix is tightly bonded. As such, it has the least solubilized lignin (329.12 ng/mg). Again, no statistical difference was observed on the HMF, which may be due to the low temperatures used in this experiment.

Table C.3 Lignin and furfurals in radio frequency pretreated barley straw grind.

Tempt. (^{o}C)	NaOH conc. (%)	Hammer mill screen size (mm)	HMF (ug/mg)	FUR (ug/mg)	Lignin (ng/mg)
40	1	1.6	2.49 (0.19)	30.37 (16.05)	464.79 (50.67)
50	1	1.6	2.25 (0.25)	36.65 (8.64)	525.38 (151.62)
60	1	1.6	2.68 (0.16)	43.09 (0.12)	550.60 (79.84)
40	0.5	1.6	2.53 (0.12)	35.24 (10.78)	379.50 (23.33)
50	0.5	1.6	1.88 (1.32)	27.20 (9.54)	385.83 (49.43)
60	0.5	1.6	2.71 (0.21)	40.70 (6.00)	415.99 (66.88)
40	1	0.8	2.43 (0.16)	37.49 (5.85)	545.15 (32.03)
50	1	0.8	2.51 (0.02)	34.86 (12.89)	574.01 (16.94)
60	1	0.8	2.64 (0.63)	33.40 (16.03)	507.82 (17.76)
24	1	0.8	2.43 (0.15)	35.50 (3.48)	587.18 (98.22)
24	0.5	0.8	2.69 (0.03)	30.41 (21.84)	398.67 (58.95)
24	0	0.8	2.70 (0.35)	43.17 (4.91)	329.12 (33.01)

Values in parentheses = standard deviation; n = 2, HMF = 5-hydroxyl-methyl furfural and FUR = furfural.

Table C.4 indicates the average sum of the monomeric reduced simple sugars (xylose, arabinose, glucose, galactose, and mannose) measured using UPLC. The hemicellulose was calculated from the sum of xylose, arabinose, galactose, and mannose. While cellulose was assigned to glucose. This is because the UPLC has no capability to separate the glucose from cellulose and hemicellulose. The cellulose from the raw sample is higher because there was no initial degradation of the sugar unlike the pretreated samples. This implies that there is trade off during pretreatment of biomass: there is deconstruction and breakdown of lignin as well as degradation of the energy potentials (cellulose and hemicellulose).

Table C.4 Cellulose and hemicellulose content of radio frequency pretreated barley straw grind

Tempt. (^{o}C)	NaOH conc. (%)	Hammer mill screen size (mm)	Cellulose (%)	Hemicellulose (%)
40	1	1.6	11.57 (1.60)	13.61 (2.90)
50	1	1.6	14.77 (0.49)	11.24 (2.04)
60	1	1.6	12.00 (1.71)	13.04 (1.82)
40	0.5	1.6	12.08 (2.40)	11.56 (1.23)
50	0.5	1.6	10.63 (6.55)	10.45 (7.19)
60	0.5	1.6	12.02 (1.88)	14.07 (3.82)
40	1	0.8	13.94 (5.02)	17.47 (7.16)
50	1	0.8	12.74 (0.70)	13.86 (2.58)
60	1	0.8	13.94 (5.02)	15.50 (3.68)
24	1	0.8	10.17 (0.860	13.72 (1.01)
24	0.5	0.8	11.00 (1.35)	14.80 (0.50)
24	0	0.8	16.46 (3.23)	14.28 (1.65)

Values in parentheses = standard deviation; n = 2

C.4.2 Durability

Table C.5 shows the durability values of the pellets. Lignin plays a very vital role in the binding of biomass particles. RF pretreatment with 1% NaOH concentration produced pellets with higher durability. This is as a result of the pores which has been created on the lignin matrix during the RF pretreatment process, and subsequently made the binder (lignin) easily accessible during the densification process to produce durable pellets. Figure C.2 shows the pellets produced using the single pelleting Instron machine. The high lignin value in Table C.3 for samples pretreated with 1% NaOH confirmed that the lignin in pretreated samples were available for binding of particles. The statistical analysis performed indicated that concentration has significant effect on durability of the pellets.

Table C.5 Durability, tensile strength, and fracture load of pellets made from radio frequency pretreated barley straw grind

Temperature (^{o}C)	NaOH concentration (%)	Hammer mill screen size (mm)	Durability (%)	Tensile strength (MPa)	Fracture load (N)
40	1	1.6	71.66 (9.13)	1.17 (0.43)	27.12 (10.12)
50	1	1.6	76.27 (10.55)	1.21 (0.34)	28.12 (7.94)
60	1	1.6	74.18 (8.81)	1.29 (0.51)	30.08 (11.85)
40	0.5	1.6	68.29 (13.73)	0.87 (0.24)	20.19 (5.51)
50	0.5	1.6	61.02 (9.54)	0.86 (0.41)	19.96 (9.56)
60	0.5	1.6	66.94 (12.22)	0.84 (0.50)	19.61 (11.59)
40	1	0.8	78.69 (14.98)	1.07 (0.48)	24.88 (11.11)
50	1	0.8	73.50 (9.95)	1.17 (0.45)	27.12 (10.43)
60	1	0.8	73.08 (5.98)	0.95 (0.28)	22.03 (6.51)
24	1	0.8	67.91 (9.52)	1.15 (0.39)	26.67 (9.11)
24	0.5	0.8	65.74 (4.89)	0.46 (0.17)	10.59 (3.92)
24	0	0.8	63.83 (10.12)	0.29 (0.10)	6.78 (2.29)
24	0	1.6	67.79 (8.98)	0.33 (0.07)	7.79 (1.63)

Values in parentheses = standard deviation; n = 10

Figure C.2 Pellets produced from the pretreated biomass using single pelleting Instron machine.

C.4.3 Tensile Strength

Table C.5 shows the tensile strength values of the pellets. Again RF pretreated with 1% NaOH concentration produced pellets with higher tensile strength. The already released or broken down lignin binder helped in the particle binding mechanisms and behavior. The 1% NaOH RF pretreated sample enhanced the creation of the mechanical interlocking of the biomass particles and supported the strength of the bonds between the adhering partners upon application of pressure and temperature. The linear regression analysis shows that concentration and particle size has significant effect on tensile strength and fracture load.

C.4.4 Pellet Density and Dimensional Stability

Table C.6 shows the density and % density change values of the pellets. Samples pretreated with 1% NaOH concentration has higher density because the released lignin binder enhanced the mechanical interlocking of the particles, increases adhesion among the biomass particles, favors the generation of the intermolecular bonds within the contact area of the biomass particles (Tabil 1996). When biomass is subjected to heat, lignin tends to become soft, melts and exhibits thermosetting binder resin features (Van Dam 2004a) to produce pellets with high density and dimensional stability. Concentration also has significant effect on density. The positive and negative % density change corresponds to diametric and longitudinal contraction and expansion, respectively, of the pellets after two weeks of storage.

Table C.6 Pellet density and change during 2 weeks of storage of pellets from radio frequency pretreated barley straw

Temperature (^oC)	Concentration (%)	Particle Size (mm)	Density1 (kg/m^3)	Density2 (kg/m^3)	% change in density
40	1	1.6	1234.80 (22.95)	1245.34 (21.22)	0.85
50	1	1.6	1270.88 (35.51)	1270.38 (17.96)	-0.04
60	1	1.6	1230.48 (26.60)	1205.11 (22.24)	-2.11
40	0.5	1.6	1138.71 (53.15)	1143.61 (39.84)	0.43
50	0.5	1.6	1065.50 (11.80)	1070.68 (18.00)	0.48
60	0.5	1.6	1085.60 (23.82)	1088.79 (23.51)	0.29
40	1	0.8	1192.95 (20.00)	1129.02 (180.43)	-5.66
50	1	0.8	1214.29 (19.96)	1202.52 (21.10)	-0.98
60	1	0.8	1190.30 (21.41)	1192.51 (11.57)	0.19
24	1	0.8	1188.58 (13.47)	1170.07 (15.32)	-1.58
24	0.5	0.8	969.34 (27.09)	973.92 (23.61)	0.47
24	0	0.8	988.47 (23.82)	1013.53 (37.36)	2.47

Values in parentheses = standard deviation; n = 10, Density1 = density of pellets immediately after the pelleting & Density2 = density of pellets after two weeks of storage.

C.5 Conclusion

The applicability of radio frequency technique as a pretreatment method of lignocellulosic biomass has been investigated. The analysis depicts that 1% NaOH concentration has a significant effect in the deconstruction of the lignocellulosic matrix. This helps in the easy accessibility and digestibility of the energy potentials and also enhances the production of better,

durable and dimensionally stable pellets. The use of NaOH during the RF pretreatment leads to increase in ash content of the pretreated samples.

Appendix D

D. Difficulties/Issues Encountered during the Steam Explosion Pretreatment (Chapter 3 and 4)

D.1 Temperature Profile during the RF Pretreatment

There were critical challenges that were encountered using the Lexan polycarbonate reactor. There was arcing and creation of hot spots due to the interaction between the compositions of the reactor, NaOH solution, and the generated electromagnetic field. This led to the generation of non-uniform heat distribution profile (Figure D.1).

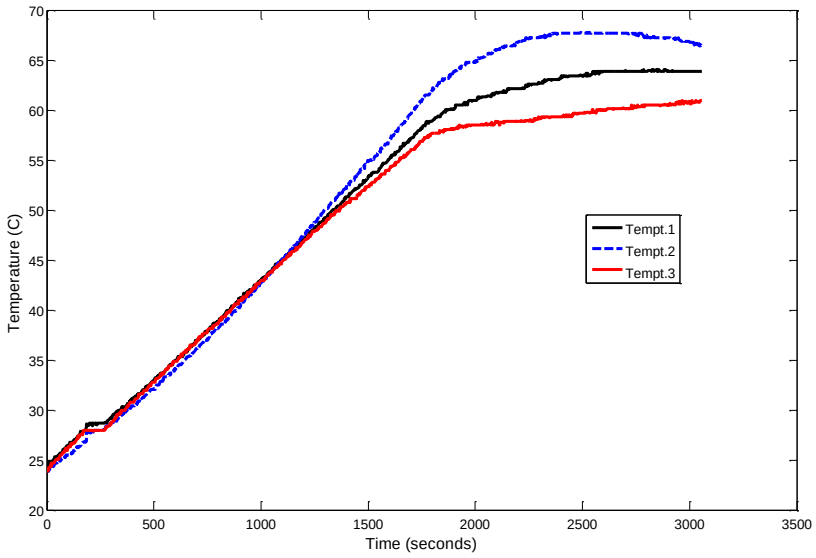


Figure D.1 Temperature profile at 60°C, 0.5% NaOH, 1.6 mm particle size, and ratio 1:6 with old Lexan polycarbonate reactor, the temperature profile is broad because of the wall and material (Lexan polycarbonate) effects.

D.2 Problems Encountered during the Steam Explosion Experiments

Due to the small inlet opening (about 10 mm) as well as the moist nature of the samples, loading the samples into the reactor was very difficult. As such, a thin rod was used to push down the samples as they were loaded into the reactor. This resulted in the loaded samples sticking to the walls of the reactor. Since the heat source of the reactor was wrapped round it, the heat transfer was by conduction which comes from outside to inside of the reactor. This resulted in the wall of the reactor having a higher temperature than the inside of the reactor.